

# A STUDY OF THE TUMBU-FLY, *CORDYLOBIA ANTHROPOPHAGA* GRÜNBERG, IN SIERRA LEONE

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## I. INTRODUCTION

Although it is more than sixty years since human Myiasis due to the larva of *C. anthropophaga* was first investigated by two French naval surgeons in Senegal, it is a remarkable fact that even to-day complete unanimity as to the mode of infection, the seasonal incidence, the natural reservoirs of the infection, and the appropriate prophylaxis, is by no means attained. It is interesting to find, in respect to this fly and also the South American fly, *Dermatobia cyaniventris*, that a disregard of native accounts has led research into side tracks which have been followed for long periods, before it was discovered that they were leading in the wrong direction. Nothing can better illustrate the manner in which this has occurred than the following extract from the excellent account of Cordylobia Myiasis given by Coquerel and Mondière (1862), who published a paper—the first so far as is known at present—on this subject. Writing of this form of Myiasis as observed by them at Portudal in Senegal, they say ‘ Cette singulière affection est connue des indigènes, qui savent très bien extraire les larves qui les tourmentent et viennent souvent se loger dans les tissus du scrotum de ces malheureux. Ils prétendent que ces vers sont produits par une petite mouche très commune à Portudal. Cette mouche pondrait ses œufs dans le sable humide, le ver y séjournerait jusqu’au moment où profitant du repos d’un homme étendu sur le sol, il s’introduirait dans la peau de sa victime.’

Unfortunately the authors were so little impressed by the probability of this suggestion that they added ‘ Il n’est pas besoin d’insister sur les détails de ce récit pour en signaler les erreurs. Il est évident que les larves du Diptère du Sénégal ont été déposés dans la peau, ou que les œufs ont été fixés a quelque poil de cette membrane dès leur origine et que les vers ne peuvent vivre ailleurs.’ The theory held by natives in Senegal in those days with regard to the mode of infection by the larvae of Cordylobia, is the same which is held to-day by natives of Sierra Leone, and it is the theory which has been proved to be, in all essentials, correct, by the accumulated research of European observers up to the present day.

In a similar way we find that the work which has been done on the bionomics of *D. cyaniventris* in South America has revealed the



interesting fact that the eggs of this species may be transported on the body of a mosquito and deposited on the animal which is to serve as the host of the larva. It has taken many years to produce the scientific evidence that such a remarkable means of infection can occur, yet the native peasants have known it for so long that their name for the larva is Gusano de Zancudo, *i.e.*, the worm of the mosquito. The suggestion of such a means of infection was treated with frank incredulity by such observers as Da Silva Arango, who refers to the peasants' belief in the transference of Dermatobia infection by mosquitoes as 'a popular error, very widely spread throughout Brazil.' This incredulity of those who first investigated the bionomics of Dermatobia had the same effect as that mentioned above, in delaying the discovery of the fact that the mosquito, *Janthinosoma lutzi*, actually does transport the eggs of Dermatobia.

The suggestion has been made by Zepeda (1913) that the larvae of Cordylobia may be carried in the same way by mosquitoes; we have found no evidence of this.

The accurate knowledge displayed by the intelligent African native, the uneducated native who uses his powers of observation to the fullest extent, is often impressive. The Protectorate natives of Sierra Leone quite commonly make for example the nicest distinction between the bionomics of the testaceous flies, *C. anthropophaga* and *A. luteola*. They recognise that the flies are very similar to each other in appearance, and that each produces eggs out of which larvae proceed; they are perfectly aware, however, that of one, the larva lives in the tissues of the host and produces boils, while of the other, the larva merely feeds on the cutaneous blood of the host and lives in the ground, only emerging at night. They can also procure samples of either larva in a short time if required to do so, the former expressed from the larval tumours in the skin of affected animals, the latter obtained from the earth on the floor of their huts by the simple procedure of sleeping on a mat on the floor and searching under the mat in the early hours of the morning. It is, therefore, no more than justice to recognise how in Myiasis, as in very many other diseases, the knowledge of the natives, acquired by the slow and painful process of racial and personal experience, has assisted the investigator to the correct solution of the problems of disease.

## II. NOMENCLATURE

From the earliest days of our knowledge of Myiasis due to *Cordylobia*, that is to say, from the time of the publication of the communication of Coquerel and Mondière in 1862 until the most recent times, when in 1914 Roubaud published his well-known treatise on Myiasis in French West Africa, very various opinions have been held as to the probable relationship which existed between larvae from diverse hosts in different parts of Africa. Coquerel and Mondière, who were not fortunate enough to rear the adult fly, considered the larva to be that of an Oestrid.

Bérenger-Féraud (1872) was successful in rearing flies which were identified by Émile Blanchard as belonging to the genus *Ochromyia*, Macq.; Blanchard called them by the new specific name, *Ochromyia anthropophaga*. Of this name Austen (1907) says, 'Since, however, no description of the fly whatever was given, *Ochromyia anthropophaga*, Émile Blanchard, is a mere *nomen nudum*, and consequently invalid.' The association of the name *Bengalia depressa* in 1891 with larvae from a human case of Myiasis has proved an additional complication, and still remains so to-day in some publications, in spite of Grünberg's work. Austen, in the paper mentioned above, gives a statement of how the larvae of human Myiasis due to *Cordylobia* came to be associated erroneously with the adult fly *Bengalia depressa*, Walk. He points out that the type of *B. depressa* is in the British Museum, and that although it is an allied, nevertheless it is a very different, insect from *Cordylobia*; he says, moreover, that the life history of *B. depressa* is as yet unknown, and that there is not a particle of evidence to prove that its larva is a subcutaneous parasite.

It was Grünberg (1903) who, after a careful examination of all the material available, both larval and adult, came to the conclusion that the fly did not belong to the genus *Ochromyia*, nor yet to *Bengalia* nor *Auchmeromyia*; but was a fly which required for its classification a new genus; he accordingly erected the genus *Cordylobia*, with the species *C. anthropophaga* (Blanch.). He gave a long and detailed description of genus and species. Dönitz (1905), in an article entitled 'Über eine neue afrikanische Fliege mit parasitisch in der Haut von Ratten lebenden Larven,' gives a description of what he considers to be a distinct species of

*Cordylobia*, and names it *C. murium*. At the same time, Dönitz reviewed the position with special attention to the ideas expressed by Grünberg and Gedoelst (1905), and shows how the latter came to speak of a *Cordylobia anthropophaga*, Grünberg. Dönitz himself proposed the name *Cordylobia grünbergi* for the East African form.

Roubaud (1914) gives reasons for deciding that *Cordylobia murium* should not be retained as a separate species, namely, that the differences claimed by Dönitz to exist are not sufficiently striking or constant to warrant the species; at the same time the name *Cordylobia grünbergi* is dismissed by him as invalid.

Two other species also are dealt with by Roubaud; of *Cordylobia praegrandis*, Austen, he says that the subsequent discovery of the male has shown that it does not belong to the genus *Cordylobia* but should be placed in the genus *Chaeromyia*, Roubaud, while *Cordylobia rodhaini* should likewise be placed in another genus. To sum up, Roubaud says there appears to be only one species of Calliphorines belonging to the genus *Cordylobia* as defined by Grünberg; that is the fly of Cayor which was first bred by Béranger-Féraud in Senegal, *Cordylobia anthropophaga*, Blanchard. Austen, however, in his summary of the situation, referred to above, ends with the remark, 'The correct designation of this highly important and much misunderstood African Muscid is, therefore, *Cordylobia anthropophaga*, Grünberg. This authoritative statement we have, therefore, accepted.

### III. GEOGRAPHICAL DISTRIBUTION

Grünberg (1903) gave as the distribution of *C. anthropophaga* a list of places, which included Senegal, South-West Africa, Gaboon, Dar-es-salam, Zambesi, Lake Nyasa, Tanga, Delagoa Bay, Bagamoyo and Durban. The distribution later given by Roubaud is from Senegal and Lake Chad to the Cape. In the map included in his work, practically the whole region of Africa, south of about 16° North latitude, is shown as infected, with the exception of the North-East area. He points out, however, that the fly is irregularly distributed and that many large areas so far appear to be free of it, or that it has not been recorded from them.



OCCURRENCE OF *C. ANTHROPOPHAGA* IN SIERRA LEONE

Many observations of the clinical effects produced by the larva in man and animals in Sierra Leone have been made in recent years, and this Colony has for many years been considered to be a favourite haunt of *Cordylobia*. Smith (1908) remarks that 'Tumbu' is a Negro-Creole word, and gives a record of his findings of the larvae in Sierra Leone. He expresses doubt as to the correctness of the accepted mode of infection by the laying of the eggs or larvae in the skin; this doubt arose from his observations of the situation of the lesions in animals. Blenkinsop (1908) noted that in Europeans the upper part of the thigh and the buttock are the favourite site for the larvae to gain an entrance, and it is a generally received opinion that the parasites are often acquired at the latrine. The West Indian troops were often affected in the axilla, and natives, in any region. It was not known in 1908 whether *Cordylobia* was oviparous or viviparous, but Austen said that in either case, since the female is undoubtedly unable to pierce the skin with her ovipositor, the larva in its earliest stage must bore its own way through the integument by aid of its mouth hooks.

Smith made observations, as we shall see later, on the age incidence of the disease in man and animals, and also succeeded in breeding out flies from larvae obtained from rats and dogs. He mentions a wild rat which had six 'tumbus' in the bare underpart of its legs and feet, which were immensely swollen.

The prevalence of *Cordylobia* Myiasis in Sierra Leone is so considerable—the parasite itself is the cause of much discomfort to man, and causes suffering and even death in animals—that we took the opportunity of studying as carefully as possible the bionomics of the fly in its various stages, and of making experiments with a view to discovering the best method of attack upon it. We have also made observations upon the morphology of the first instar which may throw some light upon the mode of skin penetration of the larvae of other forms of Myiasis, notably those due to *Dermatobia*; owing to the fact that we found that the various stages did not always correspond to previous descriptions, we have included a short description of each of the stages of the fly. It is necessary for us to refer frequently to the work of Roubaud, as his



is the most recent and at the same time the most comprehensive work done on the subject. We have been enabled, largely owing to the greater amount of material at our disposal, not only to confirm his observations in many particulars, but also to add to them. If we are compelled to differ from him in a number of points, it is due entirely to the fortunate circumstance that a larger amount of material enabled us to carry our experiments further than he was able to do.

#### IV. MORPHOLOGY AND BIONOMICS

##### (1) ADULT (See Plate XV)

The following description of the adult stage is taken from Austen (1908): 'A thick-set, compactly-built fly of an average length of about  $9\frac{1}{2}$  mm.; specimens as small as  $6\frac{1}{2}$  mm. or as large as  $10\frac{1}{2}$  mm. in length are occasionally met with. Head, body and legs, straw yellow; dorsum of thorax and of abdomen with blackish markings; wings with a slight brownish tinge. The eyes meet together for a short distance in the median line above in the case of the male, but are separated by a broad front in the female. On the dorsum of the thorax the dark markings, which are a pair of longitudinal stripes not reaching the hind margin, are covered with a greyish bloom, and, consequently, not very conspicuous; this bloom is also present on the abdomen, but here the markings are much more distinct, especially in the female, in which the third segment, as also the fourth segment with the exception of the hind margin, is entirely black or blackish. In the female, the second segment is marked with a blackish quadrate median blotch, and has a similarly coloured hind border, broadening towards the sides, while the first segment has a narrow dark hind margin. In the male, these markings are not so extensive; the dark hind margin to the second segment is interrupted on each side of the median blotch, which is triangular in shape, and there is a yellow area of considerable size on the proximal half of the third segment, on either side of a blackish median quadrate blotch; the fourth segment is similarly but less conspicuously marked.'

## HABITS OF WILD FLIES.

The adult fly material which most observers have had at their disposal has, as a rule, been obtained by the process of breeding flies in the laboratory from larvae taken from the furuncular tumours of animals. Reference in the literature to the capture of adults is extraordinarily meagre. Rodhain and Bequaert (1913) observed wild adult females flying round the cages in which animals were kept, and followed the egg-laying process. Eggs were deposited in the straw and manure in the cages; experimental animals, monkeys and guinea-pigs, placed in the cages where the wild flies had laid, became infected, as the result of the larvae which emerged from the eggs penetrating the skin. In 1911 Roubaud, at Bamako, captured alive one fertilized female which laid eggs in captivity, and which supplied the egg and larval material for his experiments.

We have been exceptionally fortunate in this respect, because we have at Freetown, Sierra Leone, notorious in the history of Tumbu disease, been able to capture many adults indoors. Not only were numerous adult females and males captured, but several of the females were either fertilized before capture, or were fertilized after capture without difficulty. A point of interest is that these captures were effected and the experiments resulting from them were carried out in the dry season, during the months November, 1922, to April, 1923; in its proper place, further reference will be made to the bearing of this fact on the seasonal incidence of Myiasis due to *Cordylobia*.

On occasions the wild flies were seen on the wing; for example on the 27th March at sunset, on a cool evening several flies were seen in the open darting about after each other and buzzing loudly; they dashed into objects blindly, and one, a female, which had injured itself in this manner was captured.

Natives were able at times to capture adults in their houses, but the construction of their houses taken in conjunction with the resting habits of the fly as observed by us, explains the lack of success which often attended the efforts of the native to capture flies. The flies captured by us were found resting on the dark green painted ceiling of the bungalow verandah; on bright sunny days as many as three or four would be found there; on cloudy days they were rarely

found. / They would remain there motionless for long periods, and only when disturbed would they fly about with great rapidity; they emitted during flight a loud buzzing noise, similar to that produced by the blow-fly; the noise ceased when they alighted again. Against the dark surface they presented a very inconspicuous appearance, and would commonly be overlooked. It was easy to understand that if this method of resting were followed on the smoky roofs of houses of native construction, the fly would be even less conspicuous. The flies were easily caught with a collecting net, and they gave the impression of being unable to see well in the day, as they allowed the net to approach close to them without taking flight. Wild flies were seen twice at night attracted by the light of a lamp on the verandah; they flew round noisily, knocking themselves against the lamp, and several had their wings scorched and fell inside the chimney. Whether these flies had come from out of doors to the light or had come to it from some resting place indoors is uncertain. It is probable the latter is the case, as after the systematic capture of all flies resting indoors in the daytime had been undertaken, no further captures were made at the lamp at night.

Wild or laboratory bred flies when placed in glass containers such as cylinders or inverted bell jars, of which the upper end was closed with cloth, rested chiefly on the cloth in the same upside-down attitude as did the wild flies on the ceiling. During the day they were rarely on the wing, but in the early morning from seven to nine, and in the late afternoon from four to six, they became very active, flying about, striking the glass sides of the vessel and buzzing audibly. At night they rested much as in the day, but the appearance of light near them at once aroused them to great activity.

#### FOOD OF ADULTS.

Roubaud observed a wild female fly feeding on sugar, on pulped fruit, and on ground soiled by urine. / We found that both males and females, whether wild or bred, fed readily on banana and pineapple, the females feeding longer and oftener; both sexes also sucked up the juice from pieces of decomposing rat liver, and less readily fresh blood of a rat from a drop exposed on a slide.

## RESISTANCE OF ADULTS IN VARIOUS CONDITIONS.

*Direct sunlight.* Three wild flies were exposed in large test-tubes to the direct rays of the sun during the hours 11 a.m. to 2 p.m. They survived only from fifteen to thirty minutes. The results of this experiment serve to explain the fact that on bright days during the hot hours the flies come indoors to rest.

*Dry heat.* Wild flies were exposed to varying degrees of heat; they were placed singly in wide test-tubes which were plugged with wool and provided with a thermometer, the tubes were placed in a water bath which was rapidly brought up to the desired temperature. A male kept for thirty minutes at 44-45° C. was still active at the end of the period. On raising the temperature rapidly great restlessness was observed at 50° C., and at 52° C. the fly dropped suddenly dead to the bottom of the tube. The experiment was repeated with two other wild flies, one male and one female. The temperature was raised from 40° C. to 47° C. in two minutes; after one minute at 47° C. both fell dead suddenly. It appears from these experiments that a temperature of about 50° C. is fatal for the fly.

*Cold.* Four laboratory bred flies, one male and three females, were enclosed in a large tube containing a slice of banana and placed in an ice chest on 16th March, 1923. After an hour in the ice chest at a temperature of from 10° C. to 6° C. all were motionless. Two were removed to room temperature and rapidly recovered; they were then returned to the ice chest. The flies at each subsequent examination were motionless, sitting sometimes on the glass and sometimes on the banana; they were not observed to feed but changed their position slightly, and recovered their feet when shaken down. On 20th March, 1923, the one male died; the three females died on 23rd, 24th and 28th March, 1923, respectively. The powers of resistance to cold and damp are, therefore, very considerable.

**OVIPOSITION.** As we have seen, Rodhain and Bequaert observed oviposition on straw and manure in animal cages. Roubaud noted that his fly laid eggs on the glass walls of a vessel and on fruit. We found that of various sites on which gravid females in captivity were given the opportunity of depositing their eggs, the one most commonly selected was dry sand which had previously been



contaminated by the excreta of animals, in this case guinea-pigs. This fact was observed on an occasion when three females were placed in a bell-jar containing a guinea-pig in order to determine whether they would deposit eggs on the animal's skin. These flies had up to this moment been lodged in a container where they had access to cardboard, cotton wool, banana, and glass on which to oviposit; none of these sites was apparently suitable for them, as they did not utilize them. Immediately on their being admitted to the bell-jar containing the guinea-pig, two, and after a short delay, the third also, set about depositing their eggs in the contaminated sand with great eagerness and rapidity; eggs were not laid by them on banana leaves, carrot or orange, which were also present. It is perhaps not without bearing on this point that three other females in which ova apparently mature were present, died in the first container without laying their eggs. On three occasions, when sand was available and utilized, eggs were also laid on sites other than sand, but only in small numbers, viz., six on a piece of black cloth, seven on the white cloth cover of the bell-jar, and eleven on cotton wool. On only one occasion was a considerable number of eggs laid on any other material than sand, when contaminated sand was available; this was a case in which wet sand had been provided for the fly; she landed on it and protruded her ovipositor, but apparently found it too wet for her, as she immediately flew off; she laid one hundred eggs in a plug of pink cotton wool which was used as a stopper to the central aperture of the white cloth cover. It appears probable that the result of this experiment has some significance in regard to the wet seasonal incidence of this form of Myiasis in man and domestic animals. Apart from these occasions eggs were always laid in the sand provided for the guinea-pigs. In numerous experiments conducted during the laying of hundreds of eggs, flies could not be induced to leave the sand on which they were laying. The guinea-pigs did not attract them, nor did they oviposit on clean cloth nor on cloth impregnated with human perspiration, the pieces of cloth being placed in their path as they were laying their eggs. Flies on the other hand would not oviposit on sand contaminated with excreta, if the sand was too moist.

*Method of oviposition.* Generally for some hours, even a day,

before egg-laying commenced, the female could be seen pushing out and withdrawing the ovipositor, and from time to time small drops of clear fluid appeared at its tip. The procedure when ovipositing in contaminated sand was uniform for all the flies observed. The fly, having alighted on the surface of the sand, and having found a suitable area, digs with the tip of the abdomen a small cavity in the sand, backing slightly and curving the abdomen downwards to enable it to do so; the ovipositor is then extruded and pushed into the sand at the bottom of the small cavity. At this time, when the ovipositor sinks into the sand, the two hind legs bring up on either side a few grains of sand against the ovipositor, which is then withdrawn. The hind legs next move rapidly in a horizontal direction to scrape a little sand over the egg deposited in the small cavity, and to smooth the surface. The fly then advances hurriedly a few steps and commences again to dig in the sand, and repeats the whole process; she does not move in a straight line for long, but turns in her tracks frequently, with the result that a small area may be very thickly sown with eggs. The movements of the fly in the later stages of egg-laying often disturb eggs previously laid by it and uncovers them, bringing them to the surface. On cotton wool the eggs were laid on strands about one quarter-inch from the surface.

*Batches and number of eggs laid.* From the fact that his fly, which laid over one hundred and fifty eggs, died after ovipositing, Roubaud concluded that *Cordylobia* cannot survive parturition; also he concluded that probably, as the number of eggs laid was much higher than what he found in the case of *Auchmeromyia*, only one batch of eggs is laid by *Cordylobia*. Our observations show that at least two batches of eggs may be laid, and that the female does not die immediately after parturition. For example, a wild fly, No. 22, was observed to lay two batches of eggs in captivity. It was captured on 29th January, 1923, oviposited on 1st February, 1923, and again on 11th February, 1923; it died on 16th February, 1923. A laboratory bred fly, No. 38, emerged from the pupa on 23rd February, 1923, and was fertilized while still unfed on the same day by a wild male; she laid the first batch of eggs on 5th March, 1923, and a second on 8th March, 1923; she died on 10th March, 1923. Another laboratory bred fly which emerged on

10th March, 1923, and was fertilized on 11th March, 1923, laid a first batch of eggs on 17th March, 1923, and a second on 20th March, 1923; she died on 24th March, 1923. The number of eggs laid in the first batch varied from two hundred and eighty-seven to three hundred, in the second batch from ninety-four to one hundred and eighty-four. It appears probable that the batch of eggs laid by Roubaud's fly was the second batch.

*Dissection of gravid females.* Several females which died without laying eggs were examined. One laboratory bred female which emerged on 20th February, 1923 and copulated on the same date died on 3rd March, 1923, without laying; she contained three hundred and four eggs; another laboratory bred female which emerged on 10th March, 1923, and was not seen to copulate, died on 24th March, 1923, containing five hundred and three eggs in different stages of development; a wild fly, with which a wild male would not copulate, died on 23rd February, 1923; she contained four hundred and four eggs.

*Rate of Oviposition.* On several occasions when females were engaged in laying in the sand, the total time taken in laying a batch of eggs was noted, and also the rate per minute. The total time taken by one fly in laying two hundred and eighty-seven eggs was thirty-three minutes; by another for one hundred and eighty-four eggs was twenty-six minutes. During the time there were several pauses of varying length, and this reduced the average number of eggs laid per minute. On the whole, however, the rate was very constant for all the flies observed. Thus, taking a total of forty-six individual minutes timed among several flies at the time they were ovipositing, the smallest number of eggs laid in a minute was five, the highest eleven, the average per minute being eight. This relatively slow process is against the idea of egg-laying on animals.

#### LENGTH OF LIFE OF FLY IN CAPTIVITY.

The longest period during which a wild fly lived in captivity was eighteen days; this was a female, which during that period laid two batches of eggs, surviving the last oviposition for five days. Several laboratory bred females lived fourteen days, but only one lived for fifteen days.



## (2) EGG

This is white in colour and measures on an average 0.8 mm. in length; it is banana shaped, being almost straight on one side and curved on the other; it tapers somewhat towards one end. On the surface there are longitudinal grooves, and there is also a fine hexagonal reticulation. In eggs from which the larva has emerged it is seen that there is near the smaller pole a longitudinal slit extending about one-third along the flattened surface; through this slit the larva has emerged.

**SITE.** The eggs were found just under the surface of the sand in which they were laid; in cotton wool also, not on the surface but about a quarter of an inch deep. The eggs adhered in most cases to particles of sand, or in cotton wool to the strands, and could not be shaken off.

**HATCHING.** For some hours before hatching the egg shows a darker patch towards the more pointed end; as the time of hatching approaches it is seen that this dark patch is in active movement, and it is recognised as the chitinous buccal armature of the larva tearing at the inner surface of the eggshell. By means of this armature the larva cuts a linear opening on the flat surface of the egg near the small pole; as soon as it is possible to do so, it pushes its cephalic end through the aperture, which it proceeds to enlarge by vigorous movements of the anterior body segments. In cases watched throughout the process, it usually took from four to six minutes from the moment when the aperture was first observed till the time when the larva had cleared itself of the eggshell.

**RESISTANCE OF THE EGG TO VARIOUS AGENTS.**

*Room temperature.* On glass the larva emerged in three days as a rule. Roubaud found a shorter period on sand, and noted that eggs on wet sand hatched somewhat later than eggs on dry sand.

*Incubator at 37° C.* If eggs were placed in watch-glasses either dry or immersed in a small quantity of water they hatched in twenty-four to forty-eight hours, the water drying up.

*Sunlight.* Exposure to the rays of the sun for one hour, whether on glass or on dry or wet sand, did not prevent them hatching within four days. Two larvae in this experiment were watched



leaving the egg; the process was short, less than a minute elapsed before the larvae were delivered from the egg, but they dragged the eggshell about for another half minute, before getting rid of it. Eggs exposed to the sun for four days did not hatch, even when subsequently removed to the shade at room temperature.

*Dry heat.* Numerous experiments were carried out with eggs in plugged tubes placed in a water bath. Exposure to temperatures of 60° C., 55° C., 50° C., and 45° C., for two minutes killed all eggs used.

*Wet heat.* Similar results were obtained by heating eggs submerged in water in tubes placed in a water bath at these temperatures for two minutes.

*Hot ironing.* Eggs were rendered incapable of hatching by passing over them lightly a flat iron at a temperature suitable for pressing clothes. The eggs were not protected by being in cotton wool, nor even by three folds of cotton cloth; they were flattened and desiccated in the process, a point of some practical importance in view of the frequently accepted theory that clothes are infected when at the laundry.

*Cold.* Eggs placed in an ice chest did not hatch in seven days, nor after removal to room temperature. Four eggs were placed in the ice chest for forty-eight hours; on removal to room temperature two emerged in three days.

Eggs dissected out of dead females did not develop in any medium, either at room temperature or at 37° C. in the incubator.

### (3) LARVA

FIRST INSTAR. Many descriptions of larvae from cases of Myiasis have been made from larvae which were in the later stages of development. It is, however, very important that not only the later instars should be examined, but that the first instar should receive attention. At this stage, in those larvae which can produce true cutaneous Myiasis, very interesting adaptive structures are found, some of which appear to render the larvae capable of penetrating unbroken skin, while others determine the ability of the larvae either to remain in situ in the skin or to penetrate further into the tissues. The structures which attract attention chiefly are the cephalo-pharyngeal skeleton and the cuticular spines. The first

stage larva of *Cordylobia* presents points of interest in respect to both these structures, as will be seen in the description given below. Phenol was used as a clearing agent.

The newly hatched larva is white in colour and is visible to the unaided eye. It measures from 0.75 mm. to 1 mm. in length; it is somewhat fusiform, tapering from the mid region towards the anterior, and to a less degree towards the posterior extremity; it is composed of thirteen segments. The first or cephalic segment is the smallest; the mouth aperture is situated near the ventral surface of this segment; the ventral surface of the segment adjacent to the mouth is yellowish, chitinized, and densely clothed with yellow spines directed backwards. On the dorsal region of the segment there are anteriorly two rounded projections, one on either side. On the posterior portion of each projection there is a minute antenna-like structure consisting of two segments: on the anterior portion is a small chitinized pit. Near the caudal margin of the segment are several rows of backwardly directed yellow spines. Segments number two to eight are covered with backwardly directed spines, almost colourless except towards the cephalic margin of each segment where the spines are distinct and more heavily chitinized, yellow or even brown in colour. Segment nine is almost devoid of spines, being provided at the cephalic margin with a single row of backwardly directed spines and at the caudal margin with a single row of spines, in this case *forwardly* directed. Segments ten and eleven have no spines at the cephalic margin, but on the caudal margin have several rows of spines directed *forwards*, the rows being more numerous on the dorsal aspect.

Segment number twelve is longer on the dorsal than on the ventral aspect; it is densely clothed all over with large, strongly chitinized and *forwardly* directed spines. These large spines directed forwards and strongly chitinized appear to act in keeping the larva in position with its posterior end at the surface of the skin. It is interesting to note that, judging from the drawings in Surcouf's (1913) article of the first instar larva of *Dermatobia cyaniventris*, a similar arrangement exists there. This segment in *Cordylobia* is furnished with several soft digital processes; of these two are visible on the dorsal surface, one on either side of the middle line, two are situated laterally on the segment,

one on each side, while two are situated on the ventral surface, one on each side of the anal orifice.

Segment number thirteen is small and has only a few sparsely distributed spines; on this segment there are four pairs of soft digital

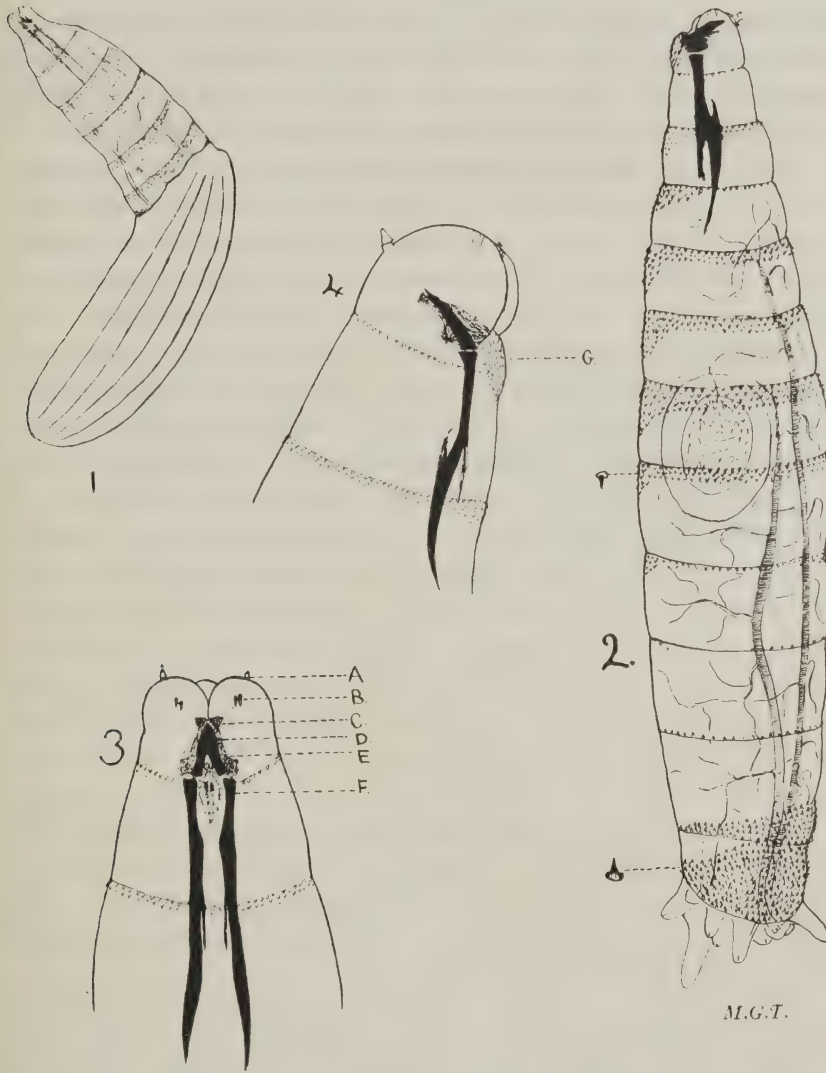


FIG. 1. (1) Egg hatching. (2) First instar larva. (3) Head of larva of first instar, ventral view. (4) Head of first instar larva, lateral view.

*A*—antenna, later papilla; *B*—chitinous pits; *C*—prestomal sclerite; *D*—median buccal spine; *E*—oral rods; *F*—cephalo-pharyngeal sclerite; *G*—ventral spiny area adjacent to mouth.

processes in addition to the tracheal tubes; the tracheal tubes open dorsally on the segment near its anterior margin on flattened eminences. The digital processes have in many cases at their tips a chitinous pit; the processes are of assistance to the larva in locomotion, and it is by means of them that the larva can attach its posterior extremity to particles of soil or other objects on which it rests and holds itself erect in the air, while waving its anterior end about in search of a host. The arrangement and position of these processes, fourteen in all, on the last two segments can be seen in figure 4.

The buccal armature or cephalo-pharyngeal skeleton is adapted for penetrating unbroken skin. It consists of a median buccal spine heavily chitinized, which articulates posteriorly with the cephalo-pharyngeal sclerites. On either side of the spine are placed the feebly chitinized oral rods (baquettes orales of Keilin). The cephalo-pharyngeal sclerites are long and slender, and have poorly developed dorsal and ventral cornua. The median buccal spine has received various names, for example, median hook, median tooth, and labral sclerite; according to Keilin, 'the median hook occurs in the primary larva of nearly all Cyclorrhaphous Diptera.' In *Cordylobia* first instar larvae, the spine when at rest is directed upwards almost at right angles to the cephalo-pharyngeal sclerite, and when in action its movements are directed upwards to lift the cuticle. Neither in its shape, nor direction, nor in its action, has it any resemblance to the mouth hooks of the second and third instars, which are, according to Lowne (1890), represented in the first instar larva by the oral rods. This median buccal spine is not present in the first stage larva of *Auchmeromyia*, which larva does not penetrate the skin; it is significant that it is present in *Hypoderma bovis* and *lineatum*, and is figured by Laake (1921), and that it persists in them up to, and including, the third instar. The retention in this case is easily understood when we consider the active migratory character of these stages.

The respiratory system consists of two main tracheal tubes which commence at the posterior stigmata on the thirteenth segment and run forward as parallel bilateral tubes; up to the tenth segment they are yellow in colour; in the region of the eleventh segment there is a transverse tube connecting them, which is also yellow in colour. Along their entire course forward they give off branches, and thus



they become attenuated anteriorly; no anterior stigmatic opening can be made out.

*Habits of the first instar larva.* The larvae remain in the situation in which they hatch out just below the surface of the sand; they are difficult to see, and even close inspection of the surface with a hand-lens may fail to reveal their presence. If, however, the container in which they are present is vibrated by tapping it, or by shaking the table on which it stands, or if the surface of the sand is disturbed by blowing on it or touching it with a needle, the larvae make their way up rapidly through the few grains of sand covering them. Similarly they come quickly to the surface and wave about, if a vessel containing hot water is brought near the surface of the sand in which they lie concealed. When the larvae are so disturbed it is easy to observe them with the naked eye; they adopt a characteristic attitude, being attached by the posterior end to a grain of sand, the rest of the body being raised in the air, and waving about actively as if seeking for something to which to attach themselves. If any object is allowed to touch the larvae they at once adhere to it and quickly crawl up on it. Camel's-hair brushes were used at first for picking up the larvae, but the larvae have the habit of at once creeping in between the hairs and disappearing from view in a few seconds; brushes were, therefore, discarded in favour of handled needles for the purpose. There is need for great care, however, that they are not injured in transferring them from the needle on to any other object such as a skin surface, as the slightest accidental pressure may render them very slow in penetrating skin or even incapable of doing so.

*Resistance of larvae of the first instar to various agents.*

**Room temperature.** Left in sand at room temperature, larvae lived without food for about nine days, as a rule; some died much earlier, and a few lived as many as fifteen days.

**On cloth.** Larvae two days hatched were taken up on cloth by laying it gently on the surface of sand containing larvae; the cloth with larvae adherent to it was kept at room temperature; the larvae lived on the cloth for nine days; a portion of wet cloth was used to pick up larvae, and allowed to dry with the larvae on it; the original condition of the cloth in this respect made no perceptible difference in the length of time larvae could live on it.

**Direct sunlight.** Larvae on a watch-glass exposed for twenty hours to open air where the sun reached them during the whole day did not die, and were capable of penetrating skin. Larvae in sand exposed to the sun for two hours in the heat of the day on one occasion only, and thereafter kept at room temperature, lived for over eleven days.

**Dry heat.** Larvae were placed in small dry tubes plugged with cotton wool; the small tube was placed in a test-tube large enough to contain also a thermometer, and the large tube was placed in a water bath. A range of temperatures and exposures was tried, and it was found that rapid definite effects were obtained at a temperature of  $50^{\circ}\text{C}$ . and above. After two minutes' exposure at  $50^{\circ}\text{C}$ . the larvae became motionless and failed to recover.

**Incubator at  $37^{\circ}\text{C}$ .** On dry sand in a watch-glass the larvae lived for three days only.

**Hot water.** In this experiment the small tubes containing the larvae to be tested were filled with water and plugged, and placed in a large test-tube half full of water, which was placed in the water bath. For this series forty-three larvae were used at various temperatures. They survived one minute at temperatures below  $48^{\circ}\text{C}$ ., but one minute at  $50^{\circ}\text{C}$ . and higher temperatures killed them. Temperatures from  $45^{\circ}\text{C}$ . to  $48^{\circ}\text{C}$ . for two minutes gave irregular results.

**Cold.** Larvae placed in a watch-glass and laid on ice, and kept there for twenty hours, became motionless but did not die; on removal to room temperature they quickly became active and were able to penetrate the skin of a guinea-pig.

**Immersion in cold tap water.** Larvae attached to needles were sunk in tubes of water, and frequently remained attached to the needle for long periods. Consecutive immersion for ten and thirty minutes produced no result; larvae immersed for ten minutes and allowed to dry, and then put in water for thirty minutes, were active at the end of the time; one made an unsuccessful effort to penetrate skin. For longer periods this method was inadequate as the larvae floated up to the surface of the water, where some of them remained active for three days. Complete immersion in tubes for long periods—in one instance up to twenty-four hours—was not fatal to them.

**Ironing.** The process of ironing was fatal to larvae in cloth, even when covered with several layers.

**Phenol.** Solutions of phenol of a strength greater than 12 per cent. killed larvae so quickly that they were unable to crawl out of the solution: solutions of less strength did not prevent them crawling out. Watch-glasses were discarded in the subsequent experiments, which were carried out as follows:—A drop of the reagent to be tested was placed on a slide, the larva placed in it, and over the larva a small square of filter paper soaked in the reagent was placed; the filter paper diminished the activity of the larva in crawling out of the fluid. Ten per cent. phenol and 5 per cent. phenol killed larvae in five minutes, while 1 per cent. sometimes failed to kill in ten minutes and frequently failed to do so in five minutes.

**Sodium hydroxide.** Solutions from 20 per cent. down were tried; all strengths down to and including 1 per cent. killed in five minutes.

**Formalin.** Five per cent. solution killed in all experiments in ten minutes, but not always in five minutes; 1 per cent. did not kill in ten minutes, but did in twenty minutes.

**Chloroform water** in the strength of 40 per cent. chloroform killed in ten minutes, but not always in five minutes.

**Calomel powder.** Larvae placed on calomel powder did not die, but moved about actively in it for an observation period of forty-eight hours.

The effect on the first instar larva of oily substance is mentioned under prophylactic experiments, and the resistance of second and third instar larva is more properly dealt with under Treatment, as these stages are already lodged in the tissues of the host.

*The skin penetrating power of first instar larvae.* Experiments made with larvae which had hatched from a few hours to as many as fifteen days previously, showed that they were capable of penetrating the healthy skin of various living animals as long as the larvae remained active. Of the fifteen days old larvae tried, only one penetrated, and that not completely; at twelve days old many larvae penetrated easily and completely. Numerous experiments were carried out on the skin of man, European and native, and on chimpanzee, dog, cercopithecus spp., cat, bush cat, guinea-



pig, wild rat and fowl; in all these cases penetration of the unbroken skin was accomplished. Larvae proved unwilling or unable to penetrate the skin of frog, lizard and python. Where penetration of the skin was successful, great variation was noticeable in the time required for the larva to conceal itself under the skin. The animals in which entry to the skin was effected most expeditiously were very young wild rats, brown or black, and in these, as in other animals, the different regions of the body offered differing degrees of resistance to the boring powers of the larvae. For example, six larvae penetrated the shaved skin of the rat abdomen in from twenty-five seconds to one minute; other six placed on the soles of the feet of the rat required from thirty seconds to two minutes. Again on the shaved skin of the thorax or abdomen of the guinea-pig, larvae penetrated in from thirty seconds to a minute and a half; larvae placed on the soles of the feet required from seven minutes to twenty-five minutes. Occasionally larvae succeeded only in partially penetrating the skin; but in no case observed, where the larva succeeded in concealing itself, did the process occupy more than half an hour.

**Method of penetration.** When an uninjured larva is deposited on the skin of an animal which is suitable, the larva quickly crawls into the nearest groove or wrinkle in the skin, puts down its head and commences to bore in. The median black mouth spine can be seen in active movement, the cephalo-pharyngeal sclerites moving in unison with it; the movement of the spine is directed to piercing the cuticle and enlarging the aperture on both sides; there is a considerable range of lateral movement of the spine, and a corresponding movement of the sclerites to which it is articulated. Once the entrance aperture is large enough to admit the cephalic end of the larva, the body very rapidly insinuates itself under a thin tunnel of cuticle; the rapidity depending on the thinness and softness of the skin. The action of the mouth-parts and the method of using them were studied very carefully in many experiments; particular attention was given to this, as the result of the observation that in the dead fixed larva of the first instar the median buccal spine was usually directed dorsally, and was not curved down ventrally as are the mouth hooks of the second and third instars. The experiments were carried out by snipping off a



very thin layer of skin from recently killed rats and placing it on a slide under the microscope and then placing on it a larva at a point where the action of the mouth-parts could be followed; if the larva entered at a point not desired, the skin could be manipulated with

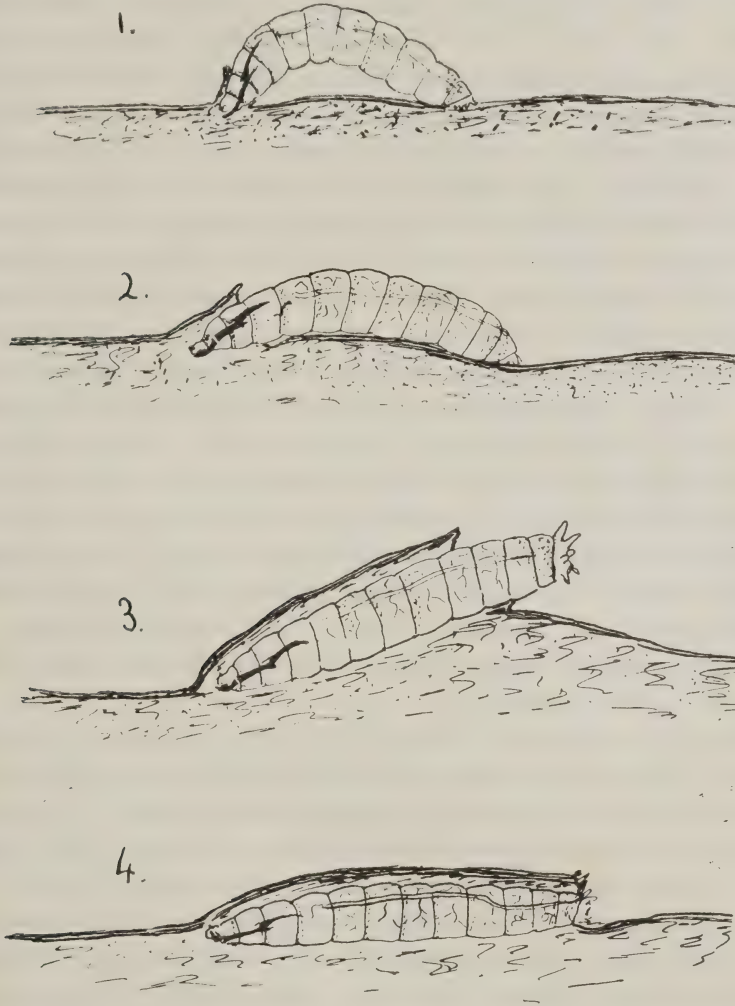


FIG. 2. First instar larva penetrating skin.

*M.G.T.*

needles, so as to bring the point of entry into view. In this way it was seen that the median buccal spine was used for penetrating the cuticle and then separating it from the subjacent tissues, by a series of punching movements directed forwards and extending

laterally until an entrance aperture was made; then followed a succession of upward movements of the mouth spine. An illustration which may serve to explain the upward movements is that they resemble the upward movements of the points of a tack-lifter, but in this case, instead of two points, there is only one formed by the buccal spine. In all cases observed, with one exception—a larva penetrated perpendicularly into the ear skin of a dead rat—the first entrance was made into the most superficial layers of the skin in a horizontal direction: the result was that as the larva lay parallel to the surface in a thin-roofed tunnel, the black buccal armature could be discerned through the layer of cuticle covering the larva. It was interesting to see that on the tail of the rat, the larvae, no matter in which direction they faced when laid upon the hairs, quickly turned themselves in such a way that they worked upwards towards the base of the tail. They followed a hair along to its origin from the skin and then proceeded to bore in, sometimes near the base of the hair, but more commonly through the skin between the hairs. The instinctive action of the larva appears to be to obtain as quickly as possible shelter and a resting place in the skin superficially, leaving the further operation of boring in deeply to be done at leisure, when it is securely ensconced and is safe from the risk of being rubbed off by the animal. The posterior segment was invariably left protruding slightly from the aperture, but could be drawn in when touched.

**Penetration of the skin of the cadaver.** Experiments were made in order to ascertain whether first instar larvae were capable of penetrating the skin of dead animals. In the first experiments the animal used was a young specimen of *Rattus rattus* which had been dead six hours. First instar larvae of various ages were placed on the skin of the ears and tail; in eleven consecutive trials the larvae eagerly attacked the skin and penetrated completely and as rapidly as they did in the living animal. In the second experiments the foot of a dead guinea-pig was used; here again penetration was accomplished with normal rapidity.

*Existence in cadaver.* Although in no case did larvae which had so penetrated complete their development, they proved capable of living in the tissues of a dead animal for two or three days; on one occasion a larva which had penetrated a cadaver reached the stage

of the first ecdysis. If an animal which had larvae of the second or third instar present on it died, the larvae as a rule at once migrated from the dead body and buried themselves in sand or soil; in the case of one guinea-pig, however, several second and also third stage larvae did not leave the host, but remained active in the tissues for twenty-four hours, before they died.

*Secondary penetration of skin.* Rodhain and Bequaert removed larvae at an advanced stage from their site in the skin of the host and inserted them into artificially made cutaneous pockets in a different host, and observed that they were capable of developing. Roubaud, as a result of his experiments, concluded that not only older larvae once removed from the host are incapable of penetrating skin afresh, but also that young larvae of the first instar are equally incapable of doing so. This he attributes to a sudden biological modification of the larva. We are in agreement with this observer as regards the lack of *penetrating* power of the second and third instar larvae, but our observations differ from his in regard to the first instar. Second and third instar larvae expressed from cavities in living guinea-pig skin made strong and often successful efforts to regain the position where they had been lodged; they made similar efforts to regain their position in tissues of dead animals on some occasions. They proved, however, quite incapable of penetrating unbroken skin, either of the same animal or of other animals. First instar larvae, however, proved capable of penetrating skin afresh, if removed from the original site within some hours of their entrance; early in the instar they are capable of re-penetration of skin, but later they are not. In Table I are given the details of some experiments.

From the table it is seen that in three experiments with guinea-pigs and rats, first instar larvae succeeded in re-penetrating skin completely; second and third instar larvae failed to do so. We consider that the factor which renders it possible for larvae early in the first instar to re-penetrate, whereas late in the first instar they are incapable of doing so, is the possession in a functional state of the median buccal spine. This apparatus is as well adapted for the purpose of skin penetration, as the mouth hooks of the second and third instars are ill adapted for the purpose.

*The effect of reagents in regard to skin penetration.* The

cadaver of the young rat was used and powders and oily substances were applied to the skin, and the larvae placed on skin so treated. It was found that French chalk, borax or calomel, delayed them, but did not prevent them penetrating the skin. Oily substances, however, had a great effect, the effect being similar for palm oil, vaseline and liquid paraffin. The larvae placed on skin treated with these did not proceed to bore into the skin; they commenced wandering about, trying to get out of the layer of liquid, and could do nothing as long as they remained in it. The disadvantages, however, are obvious, as the film of oily substance must be of considerable thickness; it is improbable that on such lines a practical prophylaxis can be evolved, as when larvae are free from the liquid they can penetrate the skin, although more slowly.

TABLE I

Giving the results of experiments to test the secondary skin-penetrating powers of the larvae of *C. antropopaga*, Grünberg.

No. of Exp.	No. of larvae	Instar # of } larvae	Animal from which removed	Animal on which tested.	Time required for penetration		Remarks
					Min.	Max.	
1	2	3rd	Brown rat	Black rat	...	...	Did not penetrate
2	2	3rd	Brown rat	Guinea-pig	...	...	Did not penetrate
3	2	2nd	Guinea-pig	Guinea-pig	...	...	Did not penetrate
4	1	2nd	Guinea-pig	Black rat	...	...	Did not penetrate
5	6	2nd	Guinea-pig	Black rat	...	...	Did not penetrate
6	1	1st	Guinea-pig	Guinea-pig	...	...	Did not penetrate (end of instar)
7	4	1st	Guinea-pig	Guinea-pig	...	...	Did not penetrate (end of instar)
8	2	1st	Guinea-pig	Guinea-pig	1 min.	3 min.	Penetrated
9	2	1st	Black rat	Black rat	8 "	...	Only one completely penetrated
10	2	1st	Black rat	Black rat	2 "	3 min.	Penetrated

*Food of first instar larvae.* First instar larvae grow slightly larger if left in contaminated sand at room temperature, but it is not possible to say whether this increase of size is due to the ingestion of food material which may be taken up in small quantities from the sand. The larvae did not show any capacity for existing



long, or developing on such substances as fruit of various kinds, pieces of muscle, blood or liver of rats. Living tissue affords their natural food.

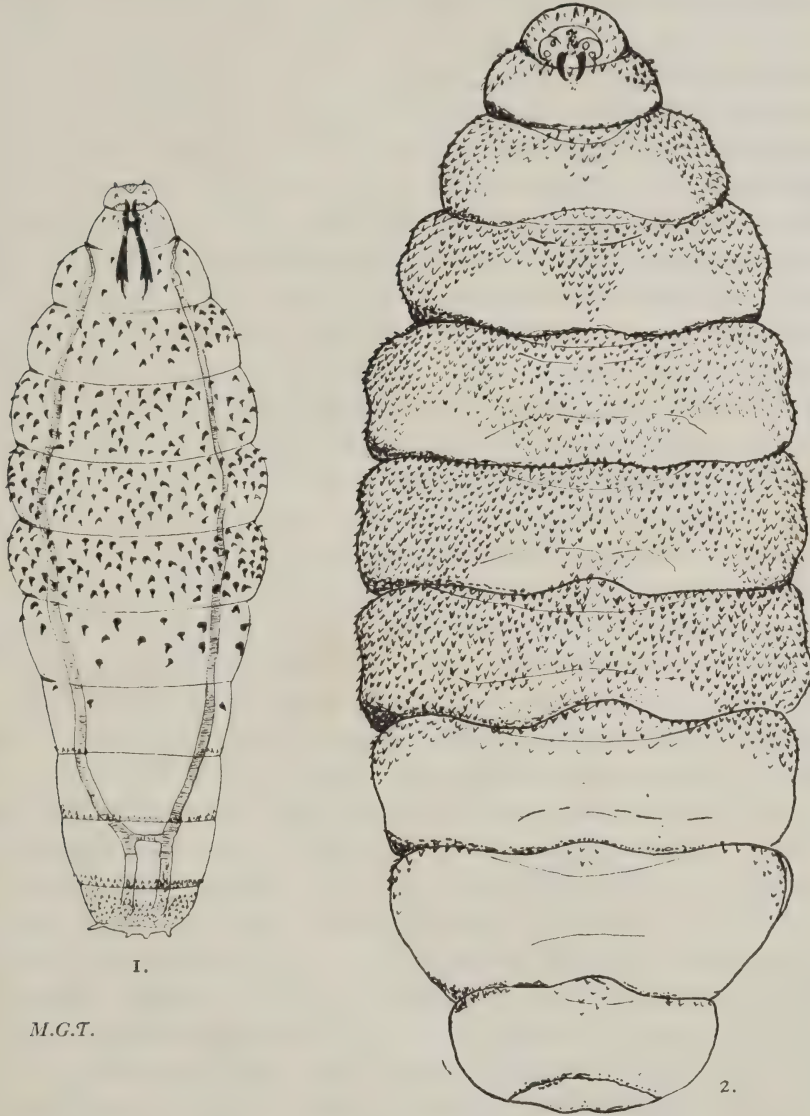


FIG. 3. (1) Larva of second instar, ventral view. (2) Larva of third instar, ventral view.

**THE SECOND INSTAR LARVAE.** This stage arises by a moult which occurs in the tissues of the host generally about the third day after penetration; the larva then measures 2.5 to 4 mm.; the

appearance and shape of the larva differ entirely from that of the first instar; whereas the first instar larva is somewhat fusiform in shape, the larva of the second instar is club-shaped; it expands quickly from the cephalic end to about the seventh or eighth segment, then narrows quickly and is more or less cylindrical to the posterior extremity. The cuticular spines in this instar are one of the most striking features, being of large size, black in colour, and distributed irregularly over the surface of the third to the seventh segments. The majority of the spines are directed backwards, but spines directed laterally or even forwards occur rarely; some of the spines are bifid. The first two segments bear a few rows of brown spines of smaller size more closely arranged on the ventral than on the dorsal surface. The segments number eight to thirteen, appear almost bare in contrast with the foregoing segments; close inspection, however, reveals the presence of a few rows of small pale spines on the posterior border of each of these segments up to the twelfth, which has numerous rows of similar spines on it. The last segment, number thirteen, which is indistinctly demarcated from number twelve, is devoid of spines; on its dorsal surface the stigmatic orifices open. There are two pairs of processes on the posterior margin of this segment, the outer pair being larger. The anal orifice which opens on the ventral aspect of the twelfth segment is provided with two lateral processes; all these processes are small, compared with the corresponding processes of the first instar. The cephalic segment viewed from the dorsal aspect, presents two rounded eminences, separated from each other by a shallow mesial sulcus. Each eminence has on its upper surface two structures, papilla-like; around the base of each is a brownish ring of chitin, and in the substance of each papilla are what look like delicate chitinous tubes opening on the surface. The papillae are situated one posteriorly and the other anteriorly on the rounded eminence, and each arises in connection with a goblet-shaped structure from the lower end of which a narrow cord passes backwards. On the ventral aspect of the segment the tips of the two black buccal hooks protrude; external to them on either side is a yellow ridge bearing small spines.

The mouth parts consist of two black hooks strongly curved ventrally, in contrast with the median spine of the first instar.

Posteriorly the mouth hooks articulate loosely with a hypostomal sclerite consisting of short rods united by a transverse bar. This sclerite articulates in turn posteriorly with the pharyngeal sclerites which pass backwards, reaching the middle of the third segment in extended specimens.

The respiratory system consists of two main longitudinal tracheal tubes which pass forwards from the posterior stigmata on the thirteenth segment to the posterior lateral border of the second segment, where they end in the anterior stigmata. The posterior stigmata consist of two curved slits, slightly oblique in direction, the concavities of the curves facing each other. The main tracheal tubes are chitinized to about the middle of the eleventh segment; just anterior to the chitinized portion a transverse tube connects the two lateral tubes. In the region of the third segment the main tubes, which have given off branches all the way forward, suddenly narrow and continue as a fine chitinized tube to the anterior stigmata, each of which consists of a fringe of finger-like processes about eight in number. Great variation in size is seen in late second instar and early third instar larvae even from the same host.

**THIRD INSTAR LARVAE.** The second ecdysis occurs in the host tissues from the fifth to the sixth days, and the resulting larva matures and leaves the host about the eighth day. The extended larva measured, when mature in rats, from 13 to 15 mm. long. Not only the measurements, but also the date on which the larva reaches maturity and the date on which the ecdysis occurs is influenced by the relative suitability of the host. The mature larva consists of twelve segments; it is roughly cylindrical in shape, its greatest width occurring at the seventh and eighth segments. The first segment is the smallest, and is frequently retracted into the second. It presents dorsally on each side a protuberance carrying two segmented papillae, the basal segment being chitinized. Ventrally on the segment are seen the free extremities of the two black buccal hooks sharply pointed and curved ventrally; on either side of the hooks there is a ridge of yellow chitinized integument bearing a row of small spines, about six in number. The mouth hooks articulate posteriorly with the hypostomal sclerite, which in turn articulates posteriorly with the pharyngeal sclerites; the posterior end of these reaches to the junction between the second and third segments. On

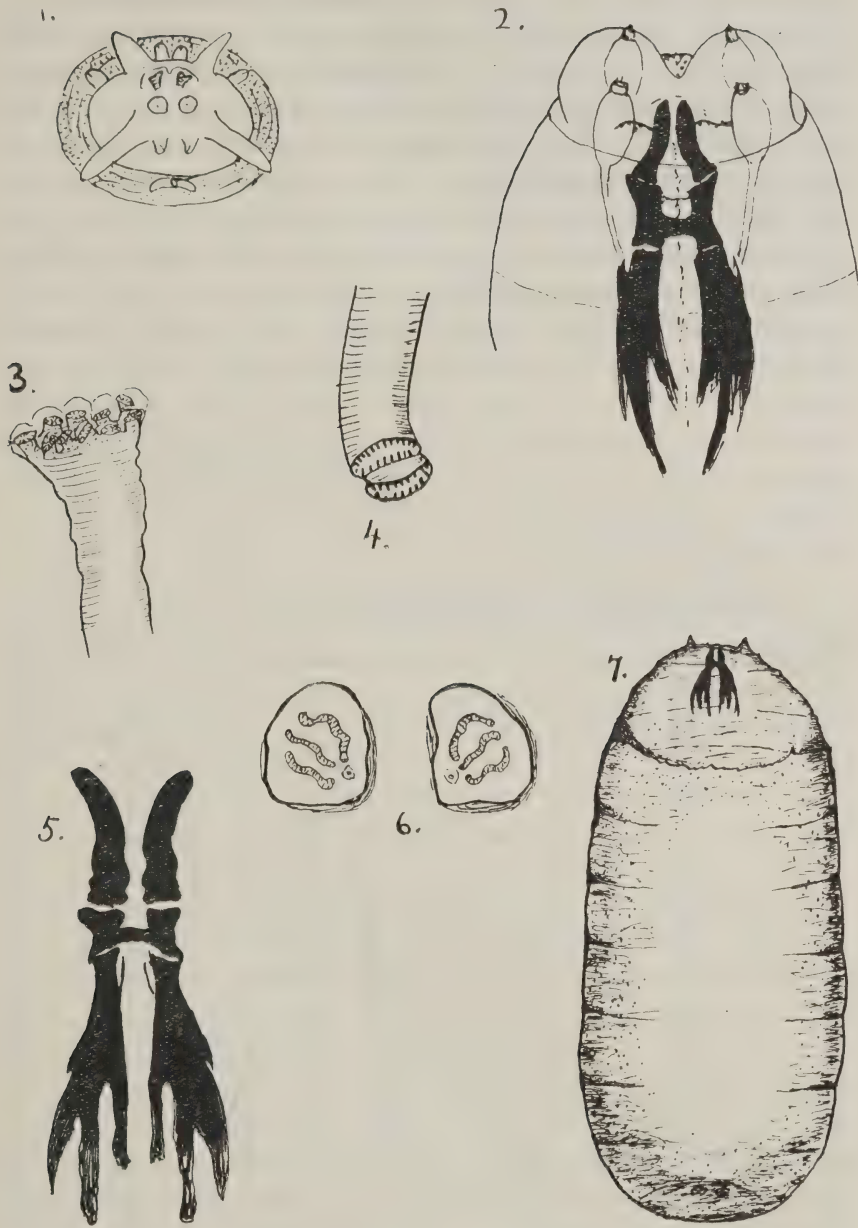


the second segment at its posterior margin laterally, the anterior stigmata open; these consist of a fringe of finger-like processes about ten in number. The twelfth segment is small; near the anterior margin of its dorsal surface the posterior stigmata open close together on rounded eminences; they consist of three sinuous slits obliquely placed; internal to the slits is a small circular opening; there does not exist in this case a definite chitinized plate on which the stigmatic slits open. The cuticle of segments number four to eleven, on the ventral surface, is thrown into folds of an irregular appearance, which are more pronounced in the middle segments, and are concerned in locomotion. Backwardly directed curved spines are present on segments two to nine, being more numerous and of darker colour on the sixth, seventh and eighth segments; on the ninth segment few spines are present, on the tenth still fewer, while the eleventh and twelfth are practically bare. The white appearance of the last four segments forms a marked contrast with the speckled black appearance of the anterior segments. The great diversity of appearance which the larva of *Cordylobia* presents in its different instars and at different stages of the same instar, has induced some observers to introduce separate names for them; this is of little assistance, and tends to add to the already sufficiently great confusion which exists in regard to the larvae of flies causing Myiasis.

#### (4) PUPARIUM

PUPATION. The process of pupation was observed in larvae which had been removed either from naturally infected or from experimentally infected animals. If mature the larvae commence to pupate within twenty-four hours; the anterior extremity becoming pinkish at first, then terra-cotta coloured; the colour extends along the body to the posterior end, and later darkens to a dark chestnut. If the larvae are immature, pupation may be delayed for a day or two, or the larvae may die and turn dark in colour; if the larva has been chloroformed or has been removed from a sloughing septic cavity, it becomes rapidly black from before backwards, the puparium fails to separate and harden, and the larva dies. The shape of the puparium is rather characteristic. It has the posterior end very squarely cut off and the sides run parallel to each other, giving an elongate oblong appearance; it tapers somewhat abruptly





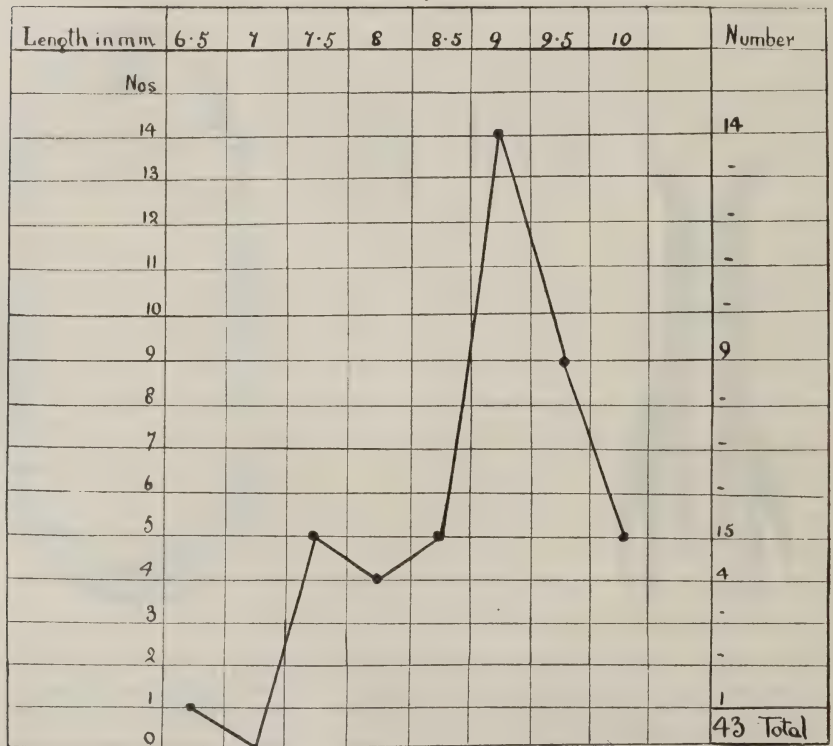
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FIG. 4. (1) Posterior view of first instar larva showing processes. (2) Cephalic end of second instar larva. (3) Anterior stigmata of second instar larva. (4) Posterior stigmata of second instar larva. (5) Cephalo-pharyngeal skeleton of third instar larva. (6) Posterior stigmata of third instar larva. (7) Puparium.

at the anterior end. On the anterior extremity of the puparium are visible two small papillae, and the posterior stigmata can be discerned in most specimens. In all cases the rings corresponding to the segments are seen, and the well defined spines of the larvae are easily made out. When the puparium is treated with phenol in order to clear it, the hard case is still more easily identified as the last larval moult, the mouth hooks and attached sclerites, the posterior tracheal tubes and stigmata, as well as the cuticular spines being readily demonstrable under the microscope.

MEASUREMENTS OF THE PUPARIUM. The smallest obtained measured 6.5 mm.; it was derived from a rat which died before the larvae were mature; the largest obtained was 11.5 mm. long; it was derived from an experimentally infected wild rat in which the larvae matured. Below is shown the distribution according to length of a series of forty-three puparia derived from the naturally infected wild rat mentioned above.

*C. anthropophaga* from a naturally infected rat which died.



GRAPH I. Showing the distribution according to length of 43 puparia of *Cordylobia anthropophaga*, Grünberg.

**SITE CHOSEN FOR PUPATION.** Larvae which had been removed from the host, or had left the host, were observed; when placed on sand which was dry they began after a short time to dig down into it, and in most cases in an hour or two they were out of sight. Sand was then provided in small tubes for each larva; the sand was so arranged that there was a damp layer of sand at the bottom of the tube of one inch in depth, and a dry layer of sand above this half an inch in depth; over fifty larvae were tested, and it was found that with a few exceptions all went right to the bottom of the tubes to pupate; the exceptions reached only the top of the damp layer. Occasionally pupation occurred on a dressing applied to an animal's limb over a tumbu lesion.

#### EXPERIMENTS WITH PUPARIA.

*At room temperature.* On 16th February, 1923, nineteen puparia derived from the larvae of a wild rat were placed at room temperature; of these seventeen emerged either on 26th or 27th February, 1923. One pupa failed to give rise to an adult, and one adult died while emerging backwards; it may be noted that on several occasions the fly was thus inverted in the puparium. Newstead (1907) drew attention to this occurrence in the case of *Auchmeromyia luteola*.

*In the ice chest.* On 16th February, 1923, twelve pupae from the same source were placed in an ice chest. None of these had emerged by 9th March, 1923; they were then placed at room temperature, and of the twelve, nine emerged on 14th March, 1923; the other three failed to emerge by 10th April, 1923, when the experiment was terminated. The resistance of the fly in the pupal stage to cold is of interest, as is also the marked prolongation of the pupal stage under these circumstances.

*In Incubator at 37° C.* On 16th February, 1923, twelve pupae from the same source were placed in the incubator at 37° C.; on 2nd March, 1923, none had emerged; eight were removed from the incubator and placed at room temperature, four being left in the incubator. Of the eight removed, four were kept dry and four were kept moist. On 9th March, 1923, none of the twelve had emerged; the four remaining in the incubator were placed at room temperature, dry. On 10th April, 1923, none of the twelve had emerged and the experiment was terminated. The resistance of the fly in

the pupal stage to dry heat is therefore small. A similar observation has been made by Dove (1918) on the resistance to heat of the pupae of *Gastrophilus haemorrhoidalis*.

*Temperature and dryness.* In order to determine, if possible, whether the failure to emerge at 37° C. was due to the effect of temperature or dryness on the pupae, the following experiment was carried out with six pupae derived from a natural infection in a mongoose. Three pupae which had pupated on 19th and 20th March, 1923, were placed at room temperature in a calcium chloride desiccator. Three adults emerged on 1st April, 1923, from the three pupae.

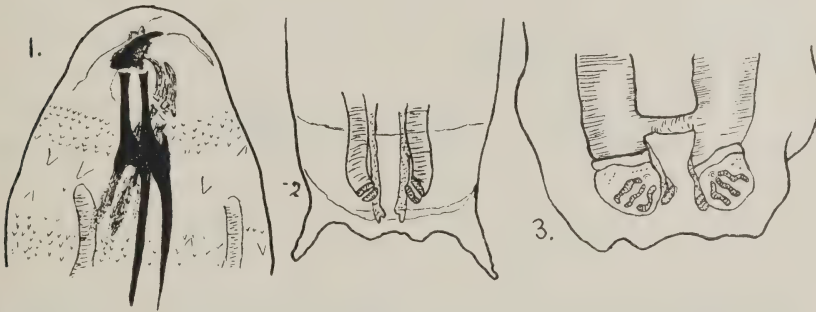
Three pupae which had pupated on 21st March, 1923, were placed in the incubator at 37° C., on 11th April, 1923, none had emerged; they were taken out and placed at room temperature; on 16th April, 1923, none had emerged and the experiment was stopped. So far as these experiments go, it appears that it is the height of the temperature and not the lack of moisture which prevents the development of *Cordylobia* at 37° C.

**METHOD OF EXIT OF FLY.** The puparium is broken at a short distance from the anterior end, and the whole cap or segments of it come off. The fly on emerging pushes vigorously through any resistant substance to escape; it was interesting to see that where puparia had been placed on a plug of wool half-way down a test-tube, plugged also at the mouth with wool, the flies which emerged most frequently went downwards through the wool on which the puparium was lying so that finally they arrived at the bottom of the test-tube. The newly emerged fly possesses great powers of forcing its way through such substances as sand, and cotton wool. As soon as it emerges, it sets about the operation of getting out of its immediate environment. The pressure exerted by the fly was very remarkable; in many cases two inches length of tight cotton-wool plug in the mouth of a test-tube being insufficient to prevent them escaping; in a few instances where the plugs were used, flies had flattened themselves between the plug and the glass to such an extent that they died, with the ptilinum extended in front of them and the wings still unexpanded. Dove (1918) carried out some experiments with *Gastrophilus haemorrhoidalis* puparia, which also showed the capability of the adults to push their way through



obstructing material. Thirty-two puparia were placed in moist loam at a depth of five inches; of these twenty-nine emerged normally, the adults pushing their way up to the surface.

**ECDYSIS.** The insect from the time it hatches out of the egg as a larva until it emerges from the puparium as an adult fly has undergone four ecdyses. The larva undergoes the first and second ecdyses in the tissues of the host, the first occurring from the second to the fourth days, and the second from the fifth to the sixth day. These dates were determined by removing larvae from their site in the skin of experimental animals at regular intervals after their first entrance, and examining them. The skin is cast from before backwards; the new stage emerging from the anterior end; we have been fortunate in finding larvae at various stages of the moulting process; for example, some specimens showed a condition in which



*M.G.T.*

FIG. 5. (1) First ecdysis, cephalic end. (2) First ecdysis, caudal end. (3) Second ecdysis, caudal end.

successive stages of the buccal armature were present together, while the posterior stigmata were still only in the earlier stage. Other specimens had the anterior portion of the cast skin loosened and crumpled backwards, while the posterior extremity of the cast was still firmly adherent, and showed the posterior stigmata of the successive stages co-existing (see fig. 5).

The third ecdysis occurs when the fly pupates, the larval skin not being got rid of, but remaining to form the puparium; the pupa which arises inside the puparium undergoes an ecdysis; this constitutes the fourth ecdysis. The pupal sheath cast off is found in the posterior end of the puparium when the fly has emerged.

Pupae removed from the puparium a little time before the time for the fly emerging were found to be enveloped sometimes completely and sometimes partially in the pupal sheath. In some cases it remained only as a delicate covering on the legs.

SIZE OF ADULTS. In the experiments related above, where certain pupae were subjected to the temperature of an ice chest, and in which the pupal period was thereby prolonged greatly, the size of the emerging flies was not appreciably affected. Of the flies which emerged in the experiment, twenty-two were measured: the males, ten in number, measured from 8 to 10.5 mm.; the females, twelve in number, measured from 8.5 to 10.5 mm. It is noteworthy that the size of the larva is no accurate guide to the size of the resulting puparium nor of the emerging adult; it is only a guide within rough limits. For example, two larvae gave the following figures:—

	Size of larva		Size of puparium	Size of adult
	Contracted	Extended		
1 ... ..	10.0	11.5	9.0	8.5
2 ... ..	9.5	11.0	10.0	10.5

The size of the emerging fly depended, however, on factors of which the degree of development of the larva is clearly one. Larvae which are removed early and which pupate give rise to flies which are small, while larvae which mature and pupate naturally give rise to large flies. In Plate XVI are shown two series, the first males and the second females, which demonstrate clearly the great variations in size which the fly may present.

FERTILISATION OF THE FEMALE. Copulation took place at once when recently emerged females were placed with wild male flies; it did not matter whether the females had fed or not; copulation lasted about two minutes and was repeated several times during the first day: after that it was not seen to occur; newly emerged males did not copulate readily.

## V. DEVELOPMENT OF *C. ANTHROPOPHAGA* Grün. IN ANIMALS

### (1) DURATION IN EXPERIMENTS

In the table given below are shown the various stages of the fly as it occurred in two of the experimental animals, a guinea-pig and a wild rat.

It is seen from the table that the development times in these cases were nearly the same, but the regularity with which the larvae in the rat completed the development was greater. As a rule, we found also that adults from rat puparia, as well as the puparia, were on the average somewhat larger than those from guinea-pigs.

### (2) PATHOGENICITY TO ANIMALS

IN NATURE. The animals most commonly found by us to be affected were wild rats, both brown and black; the feet, the genitals, the tail and the axillary region were chiefly involved where single larvae were present; in heavily infected animals any site was apparently suitable, including the nose. Dogs were also affected, and other animals more rarely. Lesions of the feet of sheep and goats were attributed by natives to the larva, but we did not find evidence of infection in those examined—about fifty. Various animals presented old scars and also suppurating sinuses which from the appearance might have contained larvae. The resulting lesions produced by the larvae during growth are illustrated by the case of a wild rat which had torn part of the skin off its abdomen in endeavouring to get rid of the larvae. As a rule, however, animals even with many larvae present either would not, or could not, get rid of them even when in quite accessible places. Several rats and a mongoose died apparently as the result of natural infection.

IN EXPERIMENTS. Guinea-pigs which were allowed to walk on infected sand soon showed signs of infection in the papule formation on the feet; by the third day the feet began to swell and the animals were seen biting them; in two days more great oedema of the feet was present, and in the oedematous skin the larvae could be seen; the posterior end was below the surface as a rule, and the circular aperture was smooth and polished on the margin; the

TABLE II.

Giving the development of *C. anthropophaga* in Guinea-pig and Wild Rat.

Animal	Larva Number	Days in skin	Days to pupation	Days to emergence of adult	Remarks
Guinea-pig ...	1-6	3	...	...	Removed for experiment
	7	8	...	...	Did not pupate.
	8	8	10	22	
	9	8	11	22	
	10	8	11	...	Did not emerge.
	11	8	10	22	
	12	8	10	22	
	13	8	10	22	
	14	8	11	22	
	15	8	11	22	
	16	8	10	...	Did not emerge.
	17	8	12	23	
	18	8	10	22	
	19	8	...	...	Larva lost.
<i>R. rattus</i> ...	1	8	10	23	
	2	8	10	23	
	3	8	10	23	
	4	8	10	23	
	5	8	10	23	
	6	8	10	23	
	7	8	10	24	
	8	9	11	24	
	9	9	11	24	
	10	9	11	24	
	11	9	11	24	
	12	9	11	...	Pupa preserved.



posterior stigmata are easily seen with a hand-lens at this stage, and also the more striking tracheal tubes leading forward from them, which appear as two parallel silvery lines. The cavities occupied by the larvae are of some depth, because often the posterior end of the larvae is not flush with the skin surface, but lies several millimetres below this; as the mature larva may measure up to about 15 mm., it is seen that the larva may reach with its mouth parts a point about 2 cms. from the skin surface. One guinea-pig in this experiment died as the result of the infection; the larvae were found to have penetrated to the tendons of the feet; the overlying tissues were very greatly thickened owing to the oedema. In one guinea-pig where a larva had attacked the abdominal skin, it had penetrated so deeply as to cause a tumour pushing inwards the parietal peritoneum, which was congested deeply and thickened. Wild rats and small animals frequently died as the result of infection.

In sections cut through the larva in situ in the skin of animals, where no sepsis was present there was noted only oedematous thickening and moderate round-cell infiltration of the tissue surrounding the larval body.

### (3) THE ANIMAL HOSTS IN NATURE OF *C. ANTHROPOPHAGA* Grön.

From various sources, beginning at the early observations of Coquerel and Mondière (1862) and coming down to the present day, we have collected a list of animals in which larvae of *Cordylobia* have been found. In the notes of most observers the two animals which come first are man and dog, the other hosts appearing in the records of a more limited number of observers. Neave (1912-13) mentions that dogs suffer badly, and a case in a rat. It is a very natural thing that especial note should be made of conditions affecting man, and also that any condition affecting the dog should be the subject of remark, owing to the intimate association of this animal with man. It is this natural tendency which explains, we believe, the commonly held opinion that the dog is the usual host in nature of *Cordylobia*; of this belief we shall say something later. We have found the following animals recorded as hosts:—Man, dog, guinea-pig imported and locally bred, wild rat, various

monkeys, white rat, cat, wild cat, arvicanthis, squirrel, goat and antelope. Roubaud (1914) expresses doubts about the occurrence in goats and antelopes. A case of a mule infection was reported to us, but we had not the opportunity of investigating it. We have found larvae in addition in the mongoose and chimpanzee at Freetown.

#### (4) THE MAIN NATURAL RESERVOIR OF THE INFECTION

It is a matter of great importance to determine which animal forms in nature the reservoir in which the infection is maintained and from which man derives his infection. Le Dantec and Boyé (1904) wrote, 'Le chien est l'animal de choix pour la culture de la larve.' Roubaud was struck by the fact that dogs are so often affected, as reported by previous workers and also from his own observations. He writes, of dogs, 'Ce sont ces animaux qui constituent le reservoir permanent de la myiase furunculeuse,' and that there is a striking relationship between the human cases and the presence of dogs in the immediate vicinity of those men who are infected. More than this, he concludes that the rarity or absence of dogs in any region is one of the most immediate causes of the absence of the larva from that region. 'Il semble que l'abondance du ver dans un pays soit souvent fonction de celle de la population canine.' The logical deduction which Roubaud makes is in regard to the prophylaxis of Cordylobia Myiasis; as dogs are the natural reservoir, it is against the infection which exists in dogs that man must take action. The action recommended by him consists in the regular inspection of dogs at least once a week, the removal of larvae from them, and the destruction of the larvae. He anticipates considerable results from carrying out this procedure, 'En détruisant ainsi quantité de parasites, à la source même qui les entretient normalement, on arrivera nécessairement à faire disparaître la Cordylobia des endroits qu'elle infeste, ou tout au moins à la rendre extrêmement rare.' There is no doubt but that a careful attention to the expression and destruction of larvae in dogs will diminish the numbers of Cordylobia, and here we are in complete agreement with Roubaud. Where we differ is in regard to the question of dogs being the natural reservoir of the fly; we do not believe that the dog, easily and heavily infected as it doubtless often is, plays such an important part in the maintenance and spread of infection

as to justify the optimism of Roubaud with regard to the results of his suggested method of prophylaxis. We found that in experimental trials, wild rats proved themselves more suitable hosts for the development of larvae than did even young dogs, that in nature wild rats were more frequently and severely infected than dogs, and finally we have been able to prove a close association between *Cordylobia* and wild rats, by finding in burrows of rats puparia of *Cordylobia* and rearing from them adults.

In one comparative experiment, using two pups and two medium-sized wild rats, twelve first instar larvae were allowed to penetrate the skin of the abdomen of each animal. Two larvae developed on pup number one, three on pup number two; on rat number one, eleven developed, and on rat number two, eight. The larvae from the dogs left their hosts on the tenth day, those from the rats left their hosts on the eighth day; the average size of the dog larvae was 12 mm. contracted and 14 mm. when extended, of the rat larvae was 12·5 mm. to 14·5 mm.

In nature, apart from large numbers of rats which had a few larvae in their tissues, we observed cases in which very severe infestation caused death. For example in a wild rat, *R. rattus*, nearly full grown, which was brought into the laboratory in a moribund state, there were present in various parts of its body forty-three larvae. The nose, the feet, the genitals, the tail and the general body surface were affected; the animal was in a very septic state and died soon after being brought in. Another rat presented no less than forty-one larvae, and here again the infestation resulted in death.

We have already seen that first instar larvae penetrate the skin of young rats more rapidly than that of any other animal tried; the larvae maintain themselves and develop in a higher percentage in rats than in dogs or guinea-pigs or any other animal used; the time taken by the larvae to develop in the tissues is shorter in rats than in dogs; the larvae which result from the rats are larger than from dogs. So far, then, as experiments are concerned, there is proof that the rat is a more suitable animal host for the larva than is the dog. In nature also we have found the rat more suitable.

We believe that Roubaud himself was aware of this high susceptibility of wild rats from the following three facts. The first



is, that he knew of the observation of Koch in East Africa reported by Dönitz; in East Africa, Koch found a disease in epizootic form which was killing wild rats; this disease on investigation proved not to be due to plague bacilli as first suspected, but to fly larvae present in the tissues; it was, indeed, this fly which Dönitz named *Cordylobia murium*, and which Roubaud is at some pains to prove is, in reality, *C. anthropophaga* affecting wild rats. The second fact is, that in discussing certain observations of Delanoe on *Cordylobia* in rodents, Roubaud says, 'Elles montrent que ce parasite peut trouver, en l'absence de l'homme ou de gros mammifères domestiques, son réservoir naturel chez de petits rongeurs, avec une électivité très marquée pour certaines espèces à l'exclusion des autres.' The third is, that he made the following experiments:—On page 141 of his work, his experiment B on development in the dog, resulted in twenty-two out of thirty larvae developing on the skin of a dog; he concludes, 'Le chien apparaît donc comme un hôte de choix pour l'évolution du ver du Cayor: c'est la confirmation de ce que l'on constate par l'observation naturelle.' There follows on this immediately, however, his experiment C, on development in the rat; in this twelve larvae developed out of twelve tried on the grey rat (*Mus microdon*), i.e., four larvae on each of three rats. Of this he says, 'La facilité avec laquelle le ver du Cayor évolue chez le rat, fait de cet animal l'hôte qui s'indique le mieux pour l'étude du parasite.' It is remarkable that in spite of the evidence which Roubaud himself produced in the experiments given above, he should have laid such undue emphasis on the part played by dogs in this disease in nature.

##### (5) TEMPERATURE OF ANIMAL HOST IN RELATION TO INFECTION

Roubaud (1914) observed that in certain species of vertebrates there was a more or less complete immunity towards the larvae. He considers that the development of the larvae is easy in proportion as the temperature of the animal host is low. He gives the following table of rectal temperatures in support of his thesis:—

Rat	...	...	...	...	...	36°5
Dog	...	...	...	...	...	38°5
Guinea-pig	...	...	...	...	...	39°5
Pig	...	...	...	...	...	39°5
Fowl	...	...	...	...	...	42°0



He found as we have seen that, in experimental work, the rat was a favourable animal, and that the fowl was useless for the development of larvae; we saw that he regards the dog as the natural reservoir of the infection. Man, however, he regards as secondary, 'L'homme ne représente certainement qu'un hôte accidentel, chez lequel l'évolution ne se fait pas toujours.' This statement does not accord with the theory that body temperature plays a part of paramount importance in this matter, as the rectal temperature of man, 37·2, is intermediate between that of the rat and that of the dog; from the point of view of rectal temperature man should form a very favourable host. Moreover, our experiments with guinea-pigs showed that they could often be readily and severely infected, and that the infection might even be fatal.

#### (6) DISCOVERY OF PUPARIA OF *CORDYLOBIA* IN NATURE

As a result of our laboratory experiments and the observations made on rats infected in nature, we concluded that rats are of great significance in the maintenance of the infection; the larvae found in rats must, we thought, emerge to pupate in the places where rats rested; it was decided, therefore, to investigate rat burrows and search for puparia. Although rats are common in Freetown, it proved no easy task to find rat burrows which were well defined, and which at the same time were situated so as to be capable of being dug out. Dr. W. Allan, W.A.M.S., the Medical Officer of Health for Freetown, kindly rendered us great assistance in this matter, and indicated two accessible rat burrows, one on open ground; the first burrow yielded puparia of *Cordylobia*; the soil at the entrance was carefully searched before digging was begun; the puparia were found in the first foot of the burrow proper, where the soil was light, dry and friable; from nine of these puparia which were still unopened, adult *Cordylobia* emerged in the laboratory. The second burrow was in the side of a laterite wall, and one puparium was found here, but the burrow could not be followed into the wall. In addition, puparia were found near the bungalow in rat holes among the rocks, one in each of two localities; from these adults emerged. Emphasis should be placed on the fact that along the river front at Freetown, and also along the small streams through the town, the occurrence of natural hiding places among

loose boulders, clefts in the rocks and unpointed walls, makes it possible for rats to exist easily without the necessity of making definite burrows. This fact will make the thorough survey of rat holes for *Cordylobia* a difficult operation, and will also naturally render any action against them prohibitive in cost. We are of opinion that these observations not only add one to the already long list of diseases for which the wild rat may be responsible, but also demonstrate that the prophylactic measures against dog infection suggested by Roubaud are unlikely to be as effective in eliminating either the fly or the disease caused by it as he anticipates.

#### VI. THE AGE INCIDENCE OF THE DISEASE IN NATURALLY INFECTED ANIMALS

It is common knowledge among natives of Sierra Leone that young children are more often affected by Myiasis due to *Cordylobia* than are grown persons; it is also known by them that young dogs are more frequently and more heavily infested than large dogs. They are so well aware of the fatal consequences which attend infection with the larvae of young pups, that in some places it is regarded as best to drown young pups when heavily infested. Maberley (1918) refers to Dr. K. K. Cross' observation, recorded in Sir Harry Johnston's book on British Central Africa, of maggots in native children—the whole side of a child riddled with holes. Marshall (1902) says that in Salisbury one baby had no less than sixty maggots extracted from it, and that there had been several cases in which babies had had a dozen or more. Grünberg (1903), quotes the observation of Steudel, who in Bagamoyo found larvae regularly on young dogs, but not on grown dogs; in these cases Steudel thinks that the fly seems to like to lay its young on the still soft and moist skin of the new born animal. Le Dantec and Boyé (1904) say 'young dogs above all are severely attacked, and one has sometimes to remove five or six larvae daily for several weeks in succession.' Smith (1908) says 'babies at breast and carried in a cloth on their mother's back are often affected. . . . Small pups suffer more than adult dogs and the larvae are all over them.' Roubaud (1914) mentions that he and Bouet observed numerous cases among dogs in Dahomey, and that at Khombole, even in the

dry season, he found young dogs infested; he adds ' Dans le Baol, j'ai observé de véritables nids de larves chez les petits chiens. Les adultes sont d'ordinaire beaucoup moins infestés.'

#### NON-DEVELOPMENT OF LARVAE IN SKIN OF LIVING ANIMALS

Although uninjured first instar larvae penetrate with great certainty into the skin of many species of living animal, even when the larvae are many days old, it does not follow that once established in the host tissues they will develop to maturity. This is a matter of considerable significance, and a few detailed observations may be given.

#### EXPERIMENTS ON HUMAN SKIN.

*Adult European.* 30th January, 1923. A larva 0.9 mm. long was placed on the back of the first phalanx of the little finger at 11.30 a.m.; the larva moved quickly into a wrinkle and it proceeded at once to penetrate, the mouth parts moving very rapidly making an entrance wound; the body pushed quickly into the aperture with a rippling movement, starting from about the ninth segment; no sensation whatever felt. 11.40 a.m.: First sensation felt when body of larva half concealed. 11.45 a.m.: Mouth parts ceased working for a minute; at this time only two posterior segments uncovered; the median mouth spine and sclerites plainly visible through a thin covering of cuticle. 11.50 a.m.: Whole larva concealed in tunnel of cuticle. 1.30 p.m.: Definite constant itching; redness around larva and swelling at situation of anterior end.

31st January, 1923. Definite papule formed; no irritation.

1st February, 1923. Papule larger; no itching, no irritation.

The after history of this larva, as also of one which penetrated the same day on the dorsum of the second finger first phalanx, close to a hair follicle, was that the papule gradually disappeared without further itching and discomfort. Similar experiments were subsequently performed on Europeans.

*Adult Africans (Young).* In two Africans, experiments were done with four larvae on the inner side of the upper arm.

The history of all these human experiments was similar; the larvae penetrated rapidly and caused irritation and papule formation. After three days they caused no more trouble and the

papules gradually disappeared. Roubaud applied two first instar larvae to his arm, and one to that of Dr. Bouet. The three larvae penetrated normally; as a result there was a partial development only, which went on for twenty-four hours. After this period no development occurred and the small lesions healed easily.

#### EXPERIMENTS ON ANIMAL SKIN.

*Guinea-pigs.* Eighteen larvae penetrated the skin of the feet of two guinea-pigs; of the eighteen only eight developed; these were removed on the eighth day.

*Chimpanzee.* Five larvae penetrated the skin of the forearm; none developed; in this case the animal was not prevented from scratching.

*Cercopithecus callitrichus.* Three larvae penetrated the skin of the tail, but none developed.

From these experiments the information was obtained that larvae which have succeeded in penetrating skin do not always develop; and that this lack of development could not be attributed in most cases to mechanical interference on the part of the animal.

### VII. RELATIVE IMMUNITY OF OLDER ANIMALS

Numerous observers, as we have seen, have recognised the fact that young animals are more highly susceptible to *Cordylobia* infection than old animals. From what we know of the bionomics of the fly and the method by which first instar larvae gain access to the animal body, it is improbable that the comparative rarity of the disease among older animals is due to lack of opportunity of acquiring the infection. It appears clear that the failure of adult animals to show infection is due not so much to non-penetration of the larvae into their skin, as to non-development of larvae after entrance to the skin. The body is capable of resisting the process of development of the larvae but not the entrance of the larvae; the condition is one of relative immunity. If this relative immunity exists, and we have no doubt from observation and experiment that it does exist, we must endeavour to ascertain its nature. It is not an inborn hereditary immunity, because native children and the offspring of animals belonging to the country do not possess this



immunity. It is possible to suppose that it is an immunity which results from age alone, a form of immunity which it is easier to postulate than to prove. Evidence against its being an immunity which arises simply as a result of the maturity of the animal, is provided not only by the first, but by many subsequent observers. Coquerel and Mondière (1862) mention the case of an adult spaniel which was infested by a hundred larvae and died after some days; Béranger-Féraud (1872) gives an instance of a spaniel which had seventy-eight larvae, while a pup of the same breed had three hundred, and died. Again, adult immigrants to West Africa, involving many nationalities, including Europeans and, as mentioned by Blenkinsop (1908), West Indian troops, are affected by the larvae. It appears improbable that age alone confers immunity. There remains immunity acquired as the result of previous attacks of the larvae, and this we believe to be the true cause of the fact that older animals are less frequently infected than are young ones. Experimental evidence of such an acquired immunity is naturally not easy to produce, as the animals with which experiments are carried out in Tropical Africa are almost certainly partially immune; the repeated entry of larvae to the skin and their subsequent destruction by scratching and rubbing must constantly go on. The following human observations may, however, be quoted. An adult European in Sierra Leone suffered from a natural infection, nine larvae developing in the skin; thereafter he proved resistant to infection at several attempts, as shown below:—

TABLE III.

Showing the result of attempts to infect a human adult with *Cordylobia* larvae.

Date		Number of larvae which penetrated skin		Site in body	Result
September, 1922	...	Natural infection	9	Larvae in upper arm	Removed at 6-8 mm. long
January 30, 1923	...	Experimental infection	2	Fingers	Death of larvae.
February 18, 1923	...	Experimental infection	4	Arm	Death of larvae.
March 7, 1923	...	Experimental infection	4	Arm	Death of larvae.
March 30, 1923	...	Experimental infection	6	Arm	Death of larvae.
April 6, 1923	...	Experimental infection	12	Arm	Death of larvae.
April 10, 1923	...	Experimental infection	4	Arm	Death of larvae.
April 23, 1923	...	Experimental infection	5	Arm	Death of larvae.

Certain animals gave suggestive results. Two dogs which had been infected on the abdomen with partial success, resisted infection at a subsequent date with eighteen larvae each, all of which penetrated. Into the skin of two guinea-pigs which had recovered from an experimental infection there penetrated on 7th March, 1923, four and five larvae respectively; on 15th March, 1923, six larvae; on 20th March, 1923, six larvae; on 23rd March, 1923, six larvae; on 28th March, 1923, four larvae. Although the larvae developed for a period varying from a few hours to two days, in no case did they develop beyond the first instar. A monkey previously naturally infected on the root of the tail received on the perineum on 2nd April, 1923, nine larvae; on 8th April, 1923, six larvae; on 10th April, 1923, six larvae; no development occurred beyond a papule formation which resolved on or before the third day. These instances appear to show that there is an acquired immunity against *C. anthropophaga*.

On the other hand, we encountered certain paradoxical results, as for example, the following:—On 7th March, 1923, a Creole youth received four larvae in the upper arm; development to papules only; on 8th April, 1923, a single larva penetrated and this developed normally. Again, a small dog which had had infection and had thereafter proved resistant to several infections, at the third attempt received ten larvae on 10th April, 1923; of these three developed normally. In each of these two cases anthelmintics had been administered, to the human case betanaphthol before the last infection, and to the dog carbon tetrachloride on the same day as the infection; but whether this fact is merely coincidence it is not at present possible to say.

Against the immunity theory we have also the fact that large rats often present infection; we must conclude from this, either that such rats had not acquired immunity in their early days, or else that their immunity was broken down by some cause late in life. It is not possible, with our present knowledge, to explain why this immunity, as do all forms of immunity, breaks down on some occasions. The broad conclusion, nevertheless, is that there exists among adult animals an immunity against *Cordylobia* larvae, and we believe that the foregoing experiments point to its being an immunity acquired through previous attacks of the larvae, whether

these developed or were destroyed before developing by mechanical interference on the part of the host.

#### DURATION OF IMMUNITY

So far as we are aware, there are few facts available as yet upon this point; the following observation of Heckenroth and Blanchard (1913) has some significance and deserves mention. A European in the Congo had a fox terrier which acquired infection in October, 1911; the owner got infected in November, 1911. The owner and the dog left Africa and returned in over a year. In January, 1913, the dog became infected, and in February, 1913, the owner became infected; the immunity if established by the first attack, did not persist for much over a year at the most.

#### CUTANEOUS REACTION

In the human case, where repeated attempts were made at infection, there was a severe local reaction at each of the last six attempts; the penetration of the larvae was accompanied by great itching and was followed within a few minutes by remarkable local signs. At the point of entrance of each larva a white bleb formed and spread rapidly in all directions; the larvae were placed on an area of about two inches by three, and yet in all cases within fifty minutes the white urticarial wheals had coalesced and produced an irregular swelling about three inches in diameter and raised in the centre about a quarter of an inch; around the white area was a zone of deep congestion which faded away at the margins. The rather tense white swelling stood out in a striking manner against the red background; very considerable itching was felt over the whole area affected, while at the same time a slight pricking sensation caused by the larvae boring was experienced. By next day the swelling, redness, and to a great extent the itching, had subsided.

Hadwen and Bruce (1917) made observations on Anaphylaxis, in cattle and sheep, produced by the larvae of *Hypoderma bovis*, *H. lineatum*, and *Oestrus ovis*. Intravenous injection of larval extracts in saline solution produced death rapidly in some animals, severe reactions in others, while in some it produced no ill-effect. The preliminary sensitisation was not experimental but natural, and



was attributed to the excretions of the larvae; in one case there was evidence of the sensitiveness being inherited. Anaphylaxis was only found to occur when larvae were broken in an animal, or when a dose had been injected. An ocular reaction occurred in sensitive animals when a drop of juice from a larva was placed on the conjunctiva. The cutaneous reaction in our case, while apparently anaphylactic in nature, resulted not as in Hadwen and Bruce's experiments from the injection of extracts, but from natural penetration of larvae into the skin, the larvae being uninjured.

These observations which we have made on immunity appear to us of great importance, not only in so far as concerns *Cordylobia* Myiasis, but still more in relation to the Myiasis caused by *Dermatobia* and *Hypoderma*, in which cases enormous loss is suffered year after year on account of damage to hides. The development of some method of immunizing cattle against the attacks of the larvae would result in a great increase of value of hides from countries which are affected by such larvae. The remarkable thing is that in South America, in the midst of a country in which cattle are severely affected by the larvae of *Dermatobia*, there actually exists a breed of cattle—Antioquia—which enjoys a relative immunity. We are indebted to Mr. M. T. Dawe, Commissioner of Lands and Forests in Sierra Leone, for showing us his photographs of this breed and for much interesting information concerning it. We feel convinced that a further study of this subject, with a view to discovering some means of artificially producing a definite immunity would well repay the trouble and expense involved.

#### VIII. SEASONAL INCIDENCE OF *C. ANTHROPOPHAGA* Grün.

The consensus of opinion of previous observers appears to be that the wet season is the season of prevalence of infection with *Cordylobia* larvae. Coquerel and Mondière (1862) stated that in the month of July, in Senegal, after the commencement of the rains, many cases of these larval parasites occurred. Le Dantec and Boyé (1904) say that in French Guinea, the adult appears in the beginning of the wet season, disappears abruptly in October, only to reappear next year with the first rains. Rodhain and Bequaert



(1913) mention a series of animals which are affected with larvae at Katanga, all in the wet season. Roubaud (1914) refers to Béranger-Féraud's observation that in human beings this form of Myiasis occurs in July. Howard (1912-13) says that the larva is very abundant during certain seasons in the Transvaal. The infection of human beings is considered by Roubaud to result from close association of dogs with men, the fly being primarily attracted by the dogs. Our experience as regards adult flies is not in agreement with that of Le Dantec and Boyé; during the dry season, as we have shown, the adult fly is not rare in Freetown; we captured over a hundred wild specimens, both male and female; fertilization and oviposition occurred constantly during the dry season. There does not seem to be any reason why infection of man should not occur in this season if the theory that dogs are the attraction which brings the fly near man, and form the main reservoir, were correct. That these flies were not, in this case, attracted by dogs is evident, because there was no dog present in the bungalow; it was equally clear that they were not attracted by the latrines, as in no case was a fly ever captured or seen there; nor were they attracted by the clothes accumulating for the laundry, which they were never seen to approach. They appeared not to have come in to lay their eggs, because males as well as females came in, in about equal numbers and because no female which was captured was actually ready to lay eggs when it came indoors. The correct explanation probably is, in accordance with the experimental evidence on the effects of sunlight, that the flies were simply taking shelter from the heat of the sun; we have pointed out that on dull days they did not come in. The source from which these flies came was, we think, the rat holes in the rocks adjacent to the bungalow, where, as we have pointed out, puparia were found. Rats captured near the bungalow were frequently infected, and sometimes heavily.

It might be argued that, although in this case the flies were not attracted by the presence of dogs, if dogs had been present they would have induced the flies to oviposit in the house; that might be so, but if it were the case, how can we explain the absence of human Myiasis at this time of the year in houses where dogs are kept at all seasons of the year. The fact that there is a definite

wet seasonal incidence of Myiasis in man and also dogs points to some factor at work which is independent of the presence or absence of dogs.

One explanation which suggests itself arises out of our discovery that the wild rat is the chief natural reservoir of the fly; this explanation is, that the seasonal incidence in man and also in dogs is dependent on the seasonal habits of the rat. It is commonly known that in the wet season rats congregate more closely in the neighbourhood of human habitations; this movement is due possibly to the flooding out of their burrows; this theory involves that *Cordylobia* moves with the rat, and so is brought into close association with human habitations. Another explanation is that it is simply the desire to escape from wet which makes the fly lay its eggs indoors in the wet season.

The flies came in freely, in our experience, during the dry season, but not with the idea of ovipositing; their normal place for ovipositing in the dry season is not indoors. We have seen, however, that in experiments the fly avoids wet sand and will not lay her eggs there, she will rather even lay them on cotton wool and cloth, and this was clearly done in the case of natural infection mentioned below. This fact alone might account sufficiently for the increase in human Myiasis in the rains, and also to a less extent for the increase in Myiasis of domestic animals. It is probable, however, that both factors are at work.

#### IX. MODE OF INFECTION OF MAN

The earlier erroneous ideas that the fly lays larvae or eggs in the skin of man, or that it attaches its eggs to hairs, have already been referred to. The method of infection in man is by the penetration of the first instar larvae into the skin; for this the larvae must effect contact with the skin. It is clear that there are very many ways in which such necessary contact may be brought about. It is possible, for example, that where soil or sand, especially if contaminated, is used in latrines, the female fly may deposit her eggs in the sand box; if in the act of using the sand some of it is spilt on the seat of the latrine and first instar larvae

are present in the sand, the next person to use the latrine will almost certainly have the larvae penetrate the skin. Again, if flies are hard pressed, they will lay their eggs on clean clothes, and when the larvae hatch out, if the clothes are put on, infection will occur. An interesting case is that reported to us by Dr. Wright, of Freetown, a most accurate observer, who has great experience of this disease; he was called to see a patient who had injured his shoulder; he wished to put it up at once, and for a bandage was provided with a window curtain which had been washed and ironed and had been lying in the house for some time. In a few days, only under the curtain, there developed on the patient's thorax, back and front, and on the arm, thirty-eight larvae of *Cordylobia*; the other curtain which had been lying underneath the first was examined carefully by us, but no eggs or larvae were present; there appears here the strongest evidence of the infection from clean cloth. The probability of the larvae derived from eggs laid on dirty clothing surviving the washing, exposure to the sun to dry, and subsequent ironing appears very small. Again, in view of the adult fly's dread of the bright sun and the lethal effect which this produces on the fly, it appears improbable that clothes hung up on lines to dry in the sun would have eggs deposited on them; if the clothes were laid on the ground to dry they might pick up larvae easily from the soil, but would not be so likely to pick up eggs which are lying slightly below the surface. In the houses of natives, the occupants could obtain infection by lying on infected soil, and also in any of the previously mentioned ways.

## X. SYMPTOMATOLOGY

### IN ANIMALS

The presence of one or two larvae produces little obvious distress even in small animals; when the larvae are numerous, however, there is very considerable irritation, and the obvious illness of the animal results chiefly from septic absorption combined with loss of sleep; the appetite diminishes, and the animal loses weight. Where larvae are single there is little to note beyond the localized small tumour; where larvae are close together great swelling and oedema occur, and the tissues intervening between larvae become



sloughing and gangrenous; the removal of individual larvae, then, is difficult, as the whole area of skin surrounding it may come off with them. The larvae are not always confined to the true skin, but often penetrate into the deeper tissues; in the case of some of the guinea-pigs, as we have stated, they exposed the tendons of the feet. In the abdominal wall the parietal peritoneum may be involved, giving rise to rigidity of the abdominal muscles, retraction and tenderness. In certain regions the presence of larvae produces more serious lesions than in others; the feet and scrotum easily become gangrenous; the case of one pup which became blind of an eye was reported to us; the larva had penetrated the skin before the eyes were open.

#### IN MAN

The actual penetration of the first instar larva into the skin is hardly noticeable; in some cases, like the one referred to in a previous section, an intense cutaneous reaction occurs. Roubaud observed a similar reaction in his own case, and accounted for it by saying that the larva had become contaminated from the soil in which it was. It appears to us more probable that this is a body reaction in response to the presence of a specific substance produced by the larva, possibly of a salivary nature, and that it has some connection with the marked condition of immunity which existed in one of our human cases. Such a reaction was not observed by us in the case of another European, recently arrived in this country; in this case larvae of the same batch introduced themselves into the skin without reaction and proceeded to develop. The larva developing in man is felt during the first two days or so, causing slight itching or pricking at intervals; the symptoms and signs are easily overlooked. The papule which forms, increases in size and becomes red; there is then a more or less complete cessation of symptoms for several days possibly, although the furuncular swelling increases. Then the symptoms recur with greater severity; the pain increases and becomes so sharp as to interfere with sleep. The larva becomes very active at intervals and can be seen clearly retracting into the cavity and then pushing against the margins of the aperture in the skin to increase its size. Much serous fluid may exude at this time; the skin and subcutaneous tissues have meantime



become much indurated and the area round the aperture is deeply coloured; tenderness on pressure exists; the lesion resembles a boil, for which it is frequently mistaken. Gland enlargement may occur and general symptoms, malaise and febrile reaction. The development was slow in human beings observed; in one case a larva removed on the fifteenth day in the third instar measured only 9 mm. The stage at which larvae are usually brought under notice by human beings is after the third stage has been reached, at which time the larva, in enlarging the entrance aperture preparatory to making its exit from the skin, exercises considerable force. The cavity formation is out of proportion to the size of the larva, and it appears as if the larva produced a lytic action on the tissues near its head end; the clear fluid which comes from the cavity at intervals is sometimes stained with blood and also with faeces of the larva. On removal of the larva the symptoms disappear, and healing usually occurs readily.

Nagel (1897) observed larvae in his skin in East Africa for a period of four weeks; but the record is not complete.

## XI. TREATMENT

Various methods of treatment were tried experimentally, such as the effect of tobacco smoke, insufflation of calomel powder, French chalk, dropping on tobacco juice, chloroform water, phenol solution and cresol solution in 5 per cent. strength, application of vaseline, palm oil and liquid paraffin. Simple expression was effective in removing the larvae, but often painful; removal by fine forceps was easy in the later stages if the aperture was large. Of these various methods the one finally adopted, especially for use with small larvae which cannot be removed easily with forceps, was the application of liquid paraffin and subsequent expression. Blenkinsop (1908) records the effect of a plaster of sugar and soap in causing larvae to emerge, owing to the blocking of the posterior spiracles. The natives of Sierra Leone use palm oil, the pericarp oil of *Elaeis guineensis*, with the same object; they say, however, that the Tumbu comes out at night to feed on the oil. Palm oil was tried, but was discarded owing to the colour; in its stead liquid

paraffin was used, and acted admirably even with the very small larvae. A film of paraffin is placed over the opening in the skin, any scab being first gently removed; at once the posterior end of the larva begins to come out; the film is then thickened by adding paraffin drop by drop; the larva in its efforts to reach the surface of the film makes greater movements out and in; in doing so it lubricates itself and the walls of the cavity; the superfluous paraffin is wiped off, and the two thumbs are placed a little distance on each side of the aperture and pressure inwards and downwards applied; the larva comes out slowly at first, later with more rapid movement. The larva should be destroyed. After extraction of the larva and healing of the wound, a mark remains for a long time; Fülleborn (1908) could still after ten years see the marks left on the skin.

## XII. PROPHYLAXIS

Adults should be looked for daily in houses on the ceiling of rooms and verandah, during the sunny hours of the day; any fly present should be captured in a collecting net and destroyed. All latrines should be fly-proof.

Sand and soil used for the latrine may be heated in a kerosene tin for some time before being placed in the latrine box, in this way eggs and larvae are destroyed.

In affected districts the weekly examination of domestic animals should be carefully done, and any larvae found, expressed and killed; it is most important to destroy the larvae.

Rats should be eradicated as far as is possible from houses and compounds. Those captured should be destroyed by burning before the larvae leave them.

In cases of small boils where there is doubt as to the presence of a larva, the application of a drop of liquid paraffin will cause the hind end of the larva to move actively; in any case liquid paraffin will be of great assistance in removing larvae with as little pain as possible.

Clothes lying exposed are a source of danger; it is especially underclothes and bed linen which are apt to carry infection to man; a very certain method of prevention here is to have all the clothes

ironed after washing and drying, and to store them immediately in covered receptacles.

It is advisable to have such clothes washed in the compound, and after ironing kept in drawers or suitable covered boxes; this simple precaution will prevent flies laying on them.

### XIII. COMPARISON BETWEEN *CORDYLOBIA ANTHROPOPHAGA* AND SOME OTHER MYIASIS-PRODUCING FLIES

Myiasis is a wide term embracing parasitism of very varying degrees, and involving widely different parts of the body. We shall confine our attention here to those forms of Myiasis caused by flies which in the first instar have been proved, or appear to be capable of penetrating the unbroken skin.

#### (1) *BOOPONUS INTONSUS*, Aldrich

Woodworth and Ashcraft (1923) describe a condition of Myiasis affecting the feet of carabaos and bullocks in the Philippine Islands; larvae were reared and adults bred and forwarded to Aldrich. Aldrich (1923) from three females sent to him created the new genus *Booponus* with the species *B. intonsus*. He refers to the close similarity of the adult to *Cordylobia*. The description of Woodworth and Ashcraft deals with two larval stages, but the illustration of the young larva shows that it is not like the first instar larva of *Cordylobia*; rather it resembles closely the second instar of this fly, not only in the appearance of the posterior spiracles but also in the very large and dark spines irregularly distributed over the cuticle.

It is surmised, but not proved, that the first instar larva which arises from eggs attached to hairs is capable of producing Myiasis by penetrating unbroken skin; if it appears later that this is so, we should expect, on analogy, that a first stage larva having a buccal spine will be found.

#### (2) *WOHLFAHRTIA VIGIL*, Walker

Walker (1920) described two cases of cutaneous Myiasis in infants due to larvae of this fly; evidence was given of a clinical character that these larvae appeared to have entered the unbroken

skin. Again Walker (1922) gives details of another case and also descriptions of the first instar; he says 'the median or labral hook arises from a slightly divided base, immediately in front of the pharyngeal sclerites, and is strongly decurved, the pointed apex projecting slightly from the front part of the oral aperture in the usual position.' The illustration shows well this curved hook; this character differs from the median spine in *Cordylobia* first instar larvae; the appearance of this apparatus in *Wohlfahrtia vigil* does not suggest that the mode of entering unbroken skin can be the same as in *Cordylobia*; it is possible that when skin penetration experiments are carried out, it may be found that this larva, if it can in fact penetrate healthy skin, does so by digging at once deeply into the tissue, and not, as in *Cordylobia*, by raising over its dorsal surface a thin layer of cuticle.

### (3) *HYFODERMA* spp.

Laake (1921) gives an account of the anatomy of the mouth apparatus of *H. bovis* and *H. lineatum*; he refers to the fact that Riley (1892) first described the real first stage larva of *H. lineatum* which he obtained from the egg before hatching; he mentions that Gläser (1914) and Carpenter, Hewitt, and Reddin (1914) first observed the first stage larva of *H. bovis* outside of the egg. Laake in his description and drawings shows that the median mouth spine in these larvae is retained not only during the first instar but also actually during the second and third instars, during which the larva is passing through the tissues, and is not cast off until the larva reaches the back of the host. It is interesting to note that the ventral curvature of the spine is relatively slight compared with that depicted by Walker for *W. vigil*, and resembles more the condition present in *Cordylobia*.

### (4) *DERMATOBIA CYANIVENTRIS*

Surcouf (1913) figures the first instar larva of this fly; the larva which is able to penetrate the unbroken skin is possessed of a buccal armature closely resembling that of *Cordylobia*; the large forwardly directed cuticular spines on the posterior segments which we drew attention to in *Cordylobia*, exist in this larva also.



## XIV. SUMMARY

1. The morphology and bionomics of *Cordylobia anthropophaga*, Grün., have been studied in some detail during the dry season 1922-23, in Freetown, Sierra Leone.

2. Certain new facts as regards the habits of the adult, its method of oviposition and the number of eggs laid by it are recorded.

3. In the first larval stage also, attention is drawn to certain morphological peculiarities, both in the buccal armature and in the cutaneous spinulation, which appear to have a direct and intimate connection with the process of skin penetration.

4. A direct association between *Cordylobia* and wild rats, which was suggested by field observation and laboratory experiment, has been proved to exist by the discovery of puparia of this fly in the burrow of wild rats.

5. Evidence is produced which appears to incriminate the wild rat as the main reservoir of the infection in nature, and to associate these rodents with such seasonal incidence of the disease as exists.

6. Numerous experiments were carried out both in man and animals, which add considerably to the knowledge of the mechanism of infection, pathogenicity and prophylaxis.

7. An immunity has been proved experimentally to develop against attacks of the larvae, not only in man but in animals.

8. The development of such an immunity in the case of cattle in similar forms of Myiasis is considered a possibility and worthy of investigation.

9. A comparison is made between *Cordylobia* and some other flies which cause cutaneous Myiasis.

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## EXPLANATION OF PLATE XV

*Cordylobia anthropophaga*. Adult ♂ and ♀.



del. M.G.T.



## EXPLANATION OF PLATE XVI

*Cordylobia anthropophaga*. Photograph of series of adults and puparia.



*Photo. by M. Brown*

## EXPLANATION OF PLATE XVII

Photograph of naturally infected rats (shaved with Barium depilatory powder).



photo. by M. Brown



## EXPLANATION OF PLATE XVIII

*Cordylobia anthropophaga*. Photograph of second instar larva in  
the tissues of a guinea-pig. × 8.



*Photo. by M. Brown*